Sickle Cell Anemia, a Molecular Disease[1]

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THE ERYTHROCYTES of certain individuals possess the capacity to undergo reversible changes in shape in response to changes in the partial pressure of oxygen. When the oxygen pressure is lowered, these cells change their forms from the normal biconcave disk to crescent, holly wreath, and other forms. This process is known as sickling. About 8 percent of American Negroes possess this characteristic; usually they exhibit no pathological consequences ascribable to it. These people are said to have sicklemia, or sickle cell trait. However, about 1 in 40 (*4*) of these individuals whose cells are capable of sickling suffer from a severe chronic anemia resulting from excessive destruction of their erythrocytes; the term sickle cell anemia is applied to their condition.

The main observable difference between the erythrocytes of sickle cell trait and sickle cell anemia has been that a considerably greater reduction in the partial pressure of oxygen is required for a major fraction of the trait cells to sickle than for the anemia cells (*11*). Tests *in vivo* have demonstrated that between 30 and 60 percent of the erythrocytes in the venous circulation of sickle cell anemic individuals, but less than 1 percent of those in the venous circulation of sicklemic individuals, are normally sickled. Experiments *in vitro* indicate that under sufficiently low oxygen pressure, however, all the cells of both types assume the sickled form.

The evidence available at the time that our investigation was begun indicated that the process of sickling might be intimately associated with the state and the nature of the hemoglobin within the erythrocyte. Sickle cell erythrocytes in which the hemoglobin is combined with oxygen or carbon monoxide have the biconcave disk contour and are indistinguishable in that form from normal erythrocytes. In this condition they are termed promeniscocytes. The hemoglobin appears to be uniformly distributed and randomly oriented within normal cells and promeniscocytes, and no birefringence is observed. Both types of cells are very flexible. If the oxygen or carbon monoxide is removed, however, transforming the hemoglobin to the uncombined state, the promeniscocytes undergo sickling. The hemoglobin within the sickled cells appears to aggregate into one or more foci, and the cell membranes collapse. The cells become birefringent (*11*) and quite rigid. The addition of oxygen or carbon monoxide to these cells reverses these phenomena. Thus the physical effects just described depend on the state of combination of the hemoglobin, and only secondarily, if at all, on the cell membrane. This conclusion is supported by the observation that sickled cells when lysed with water produce discoidal, rather than sickle-shaped, ghosts (*10*).

It was decided, therefore, to examine the physical and chemical properties of the hemoglobins of individuals with sicklemia and sickle cell anemia, and to compare them with the hemoglobin of normal individuals to determine whether any significant differences might be observed.

Experimental Methods

The experimental work reported in this paper deals largely with an electrophoretic study of these hemoglobins. In the first phase of the investigation, which concerned the comparison of normal and sickle cell anemia hemoglobins, three types of experiments were performed: 1) with carbonmonoxyhemoglobins; 2) with uncombined ferrohemoglobins in the presence of dithionite ion, to prevent oxidation to methemoglobins; and 3) with carbonmonoxyhemoglobins in the presence of dithionite ion. The experiments of type 3 were performed and compared with those of type 1 in order to ascertain whether the dithionite ion itself causes any specific electrophoretic effect.

Samples of blood were obtained from sickle cell anemic individuals who had not been transfused within three months prior to the time of sampling. Stroma-free concentrated solutions of human adult hemoglobin were prepared by the method used by Drabkin (*3*). These solutions were diluted just before use with the

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appropriate buffer until the hemoglobin concentrations were close to 0.5 grams per 100 milliliters, and then were dialyzed against large volumes of these buffers for 12 to 24 hours at 4° C. The buffers for the experiments of types 2 and 3 were prepared by adding 300 ml of 0.1 ionic strength sodium dithionite solution to 3.5 liters of 0.1 ionic strength buffer. About 100 ml of 0.1 molar NaOH was then added to bring the pH of the buffer back to its original value. Ferrohemoglobin solutions were prepared by diluting the concentrated solutions with this dithionite-containing buffer and dialyzing against it under a nitrogen atmosphere. The hemoglobin solutions for the experiments of type 3 were made up similarly, except that they were saturated with carbon monoxide after dilution and were dialyzed under a carbon monoxide atmosphere. The dialysis bags were kept in continuous motion in the buffers by means of a stirrer with a mercury seal to prevent the escape of the nitrogen and carbon monoxide gases.

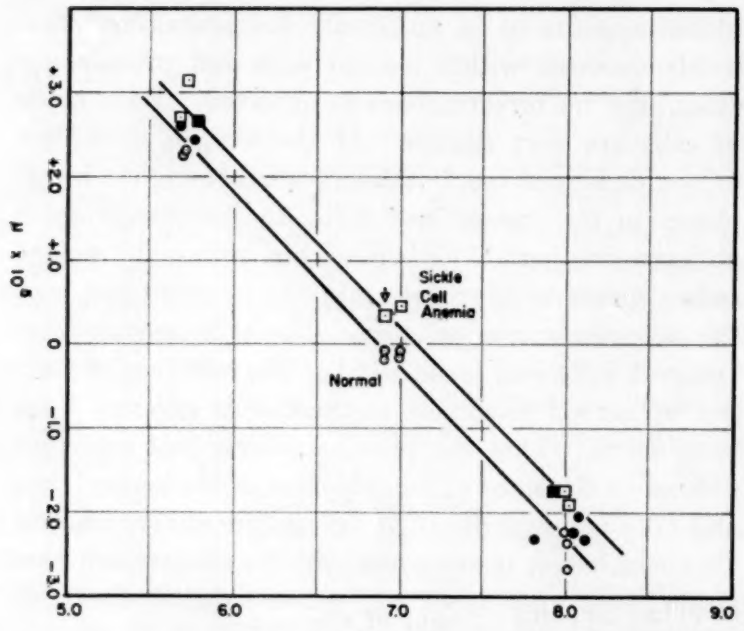

FIG. 1. Mobility(μ)-pH curves for carbonmonoxyhemoglobins in phosphate buffers of 0.1 ionic strength. The black circles and black squares denote the data for experiments performed with buffers containing dithionite ion. The open square designated by the arrow represents an average value of 10 experiments on the hemoglobin of different individuals with sickle cell anemia. The mobilities recorded in this graph are averages of the mobilities in the ascending and descending limbs.

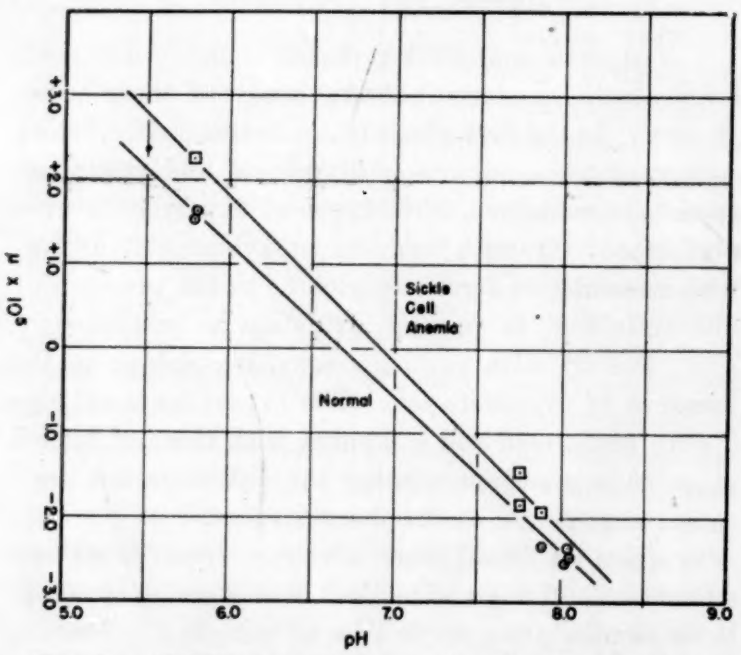

FIG. 2. Mobility(μ)-pH curves for ferrohemoglobins in phosphate buffers of 0.1 ionic strength containing dithionite ion. The mobilities recorded in the graph are averages of the mobilities in the ascending and descending limbs.

The experiments were carried out in the modified Tiselius electrophoresis apparatus described by Swingle (*14*). Potential gradients of 4.8 to 8.4 volts per centimeter were employed, and the duration of the runs varied from 6 to 20 hours. The pH values of the buffers were measured after dialysis on samples which had come to room temperature.

RESULTS

The results indicate that a significant difference exists between the electrophoretic mobilities of hemoglobin derived from erythrocytes of normal individuals and from those of sickle cell anemic individuals. The two types of hemoglobin are particularly easily distinguished as the carbonmonoxy compounds at pH 6.9 in phosphate buffer of 0.1 ionic strength. In this buffer the sickle cell anemia carbonmonoxyhemoglobin moves as a positive ion, while the normal compound moves as a negative ion, and there is no detectable amount of one type present in the other.[4] The hemoglobin derived from erythrocytes of individuals with sicklemia, however, appears to be a mixture of the normal hemoglobin and sickle cell anemia hemoglobin in roughly equal proportions. Up to the present time the hemoglobins of 15 persons with sickle cell anemia, 8 persons with sicklemia, and 7 normal adults have been examined. The hemoglobins of normal adult white and negro individuals were found to be indistinguishable.

The mobility data obtained in phosphate buffers of 0.1 ionic strength and various values of pH are summarized in Figs. 1 and 2.[5]

[4] Occasionally small amounts (less than 5 percent of the total protein) of material with mobilities different from that of either kind of hemoglobin were observed in these uncrystallized hemoglobin preparations. According to the observations of Stern, Reiner, and Silber (*12*) a small amount of a component with a mobility smaller than that of oxyhemoglobin is present in human erythrocyte hemolyzates.

[5] The results obtained with carbonmonoxyhemoglobins with and without dithionite ion in the buffers indicate that the dithionite ion plays no significant role in the electrophoretic properties of the proteins. It is therefore of interest that ferrohemoglobin was found to have a lower isoelectric point in phosphate buffer than carbonmonoxyhemoglobin. Titration studies have indicated (*5, 6*) that oxyhemoglobin (similar in electrophoretic properties to the carbonmonoxy compound) has a lower isoelectric point than ferrohemoglobin in

The isoelectric points are listed in Table 1. These results prove that the electrophoretic difference between normal hemoglobin and sickle cell anemia hemoglobin exists in both ferrohemoglobin and carbonmonoxyhemoglobin. We have also performed several experiments in a buffer of 0.1 ionic strength and pH 6.52 containing 0.08 M NaCl, 0.02 M sodium cacodylate, and 0.0083 M cacodylic acid. In this buffer the average mobility of sickle cell anemia carbonmonoxyhemoglobin is 2.63×10^{-5}, and that of normal carbonmonoxyhemoglobin is 2.23×10^{-5} cm/sec per volt/cm.[6]

TABLE 1

ISOELECTRIC POINTS IN PHOSPHATE BUFFER, $\mu = 0.1$

Compound	Normal	Sickle cell anemia	Difference
Carbonmonoxyhemoglobin	6.87	7.09	0.22
Ferrohemoglobin	6.87	7.09	0.22

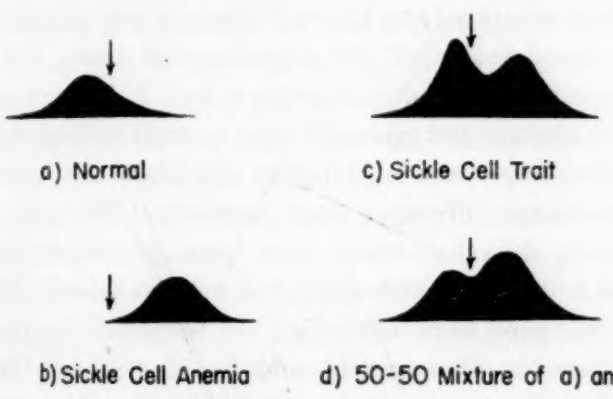

FIG. 3. Longsworth scanning diagrams of carbonmonoxyhemoglobins in phosphate buffer of 0.1 ionic strength and pH 6.90 taken after 20 hours' electrophoresis at a potential gradient of 4.73 volts/cm.

These experiments with a buffer quite different from phosphate buffer demonstrate that the difference between the hemoglobins is essentially independent of the buffer ions.

Typical Longsworth scanning diagrams of experiments with normal, sickle cell anemia, and sicklemia carbonmonoxyhemoglobins, and with a mixture of the first two compounds, all in phosphate buffer of pH 6.90 and ionic strength 0.1, are reproduced in Fig. 3. It is apparent from this figure that the sicklemia material contains less than 50 percent of the anemia component. In order to determine this quantity accurately some experiments at a total protein concentration of 1 percent were performed with known mixtures of sickle cell anemia and normal carbonmonoxyhemoglobins in the cacodylate-sodium chloride buffer of 0.1 ionic strength and pH 6.52 described above. This buffer was chosen in order to minimize the anomalous electrophoretic effects observed in phosphate buffers (*7*). Since the two hemoglobins were incompletely resolved after 15 hours of electrophoresis under a potential gradient of 2.79 volts/cm, the method of Tiselius and Kabat (*16*) was employed to allocate the areas under the peaks in the electrophoresis diagrams to the two components. In Fig. 4 there is plotted the percent of the anemia component calculated from the areas so obtained against the percent of that component in the known mixtures. Similar experiments were performed with a solution in which the hemoglobins of 5 sicklemic individuals were pooled. The relative concentrations of the two hemoglobins were calculated from the electrophoresis diagrams, and the actual proportions were then determined from the plot of Fig. 4. A value of 39 percent for the amount of the sickle cell anemia component in the sicklemia hemoglobin was arrived at in this manner. From the experiments we have performed thus far it appears that this value does not vary greatly from one sicklemic individual to another, but a more extensive study of this point is required.

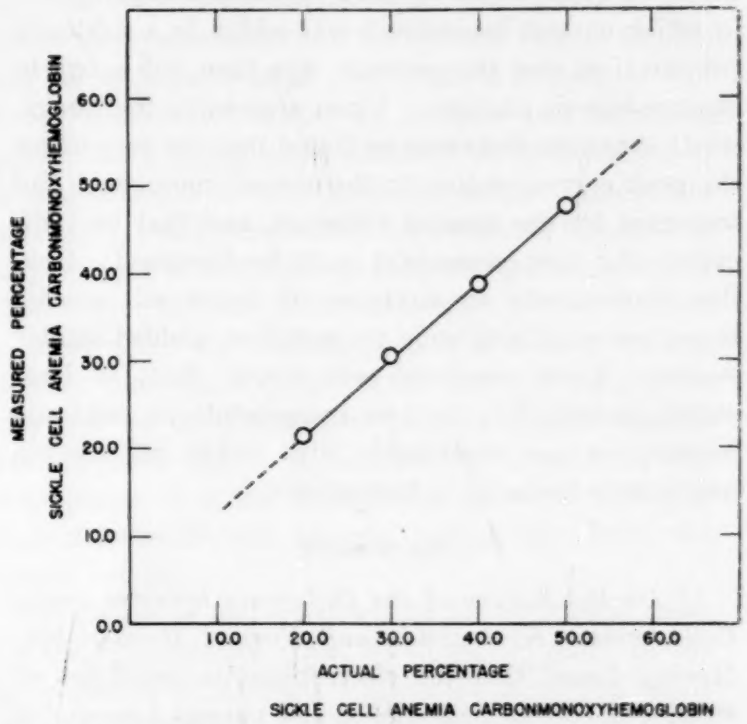

FIG. 4. The determination of the percent of sickle cell anemia carbonmonoxyhemoglobin in known mixtures of the protein with normal carbonmonoxyhemoglobin by means of electrophoretic analysis. The experiments were performed in a cacodylate sodium chloride buffer described in the text.

Up to this stage we have assumed that one of the two components of sicklemia hemoglobin is identical with sickle cell anemia hemoglobin and the other is identical with the normal compound. Aside from the

the absence of other ions. These results might be reconciled by assuming that the ferrous iron of ferrohemoglobin forms complexes with phosphate ions which cannot be formed when the iron is combined with oxygen or carbon monoxide. We propose to continue the study of this phenomenon.

[6] The mobility data show that in 0.1 ionic strength cacodylate buffers the isoelectric points of the hemoglobins are increased about 0.5 pH unit over their values in 0.1 ionic strength phosphate buffers. This effect is similar to that observed by Longsworth in his study of ovalbumin (7).

genetic evidence which makes this assumption very probable (see the discussion section), electrophoresis experiments afford direct evidence that the assumption is valid. The experiments on the pooled sicklemia carbonmonoxyhemoglobin and the mixture containing 40 percent sickle cell anemia carbonmonoxyhemoglobin and 60 percent normal carbonmonoxyhemoglobin in the cacodylate-sodium chloride buffer described above were compared, and it was found that the mobilities of the respective components were essentially identical.[7] Furthermore, we have performed experiments in which normal hemoglobin was added to a sicklemia preparation and the mixture was then subjected to electrophoretic analysis. Upon examining the Longsworth scanning diagrams we found that the area under the peak corresponding to the normal component had increased by the amount expected, and that no indication of a new component could be discerned. Similar experiments on mixtures of sickle cell anemia hemoglobin and sicklemia preparations yielded similar results. These sensitive tests reveal that, at least electrophoretically, the two components in sicklemia hemoglobin are identifiable with sickle cell anemia hemoglobin and normal hemoglobin.

Discussion

1) *On the Nature of the Difference between Sickle Cell Anemia Hemoglobin and Normal Hemoglobin*: Having found that the electrophoretic mobilities of sickle cell anemia hemoglobin and normal hemoglobin differ, we are left with the considerable problem of locating the cause of the difference. It is impossible to ascribe the difference to dissimilarities in the particle weights or shapes of the two hemoglobins in solution: a purely frictional effect would cause one species to move more slowly than the other throughout the entire pH range and would not produce a shift in the isoelectric point. Moreover, preliminary velocity ultracentrifuge[8] and free diffusion measurements indicate that the two hemoglobins have the same sedimentation and diffusion constants.

The most plausible hypothesis is that there is a difference in the number or kind of ionizable groups in the two hemoglobins. Let us assume that the only groups capable of forming ions which are present in carbonmonoxyhemoglobin are the carboxyl groups in the heme, and the carboxyl, imidazole, amino, phenolic hydroxyl, and guanidino groups in the globin. The number of ions nonspecifically adsorbed on the two proteins should be the same for the two hemoglobins under comparable conditions, and they may be neglected for our purposes. Our experiments indicate that the net number of positive charges (the total number of cationic groups minus the number of anionic groups) is greater for sickle cell anemia hemoglobin than for normal hemoglobin in the pH region near their isoelectric points.

According to titration data obtained by us, the acid-base titration curve of normal human carbonmonoxyhemoglobin is nearly linear in the neighborhood of the isoelectric point of the protein, and a change of one pH unit in the hemoglobin solution in this region is associated with a change in net charge on the hemoglobin molecule of about 13 charges per molecule. The same value was obtained by German and Wyman (*5*) with horse oxyhemoglobin. The difference in isoelectric points of the two hemoglobins under the conditions of our experiments is 0.23 for ferrohemoglobin and 0.22 for the carbonmonoxy compound. This difference corresponds to about 3 charges per molecule. With consideration of our experimental error, sickle cell anemia hemoglobin therefore has 2–4 more net positive charges per molecule than normal hemoglobin.

Studies have been initiated to elucidate the nature of this charge difference more precisely. Samples of porphyrin dimethyl esters have been prepared from normal hemoglobin and sickle cell anemia hemoglobin. These samples were shown to be identical by their x-ray powder photographs and by identity of their melting points and mixed melting point. A sample made from sicklemia hemoglobin was also found to have the same melting point. It is accordingly probable that normal and sickle cell anemia hemoglobin have different globins. Titration studies and amino acid analyses on the hemoglobins are also in progress.

2) *On the Nature of the Sickling Process*: In the introductory paragraphs we outlined the evidence which suggested that the hemoglobins in sickle cell anemia and sicklemia erythrocytes might be responsible for the sickling process. The fact that the hemoglobins in these cells have now been found to be different from that present in normal red blood cells makes it appear very probable that this is indeed so.

We can picture the mechanism of the sickling process in the following way. It is likely that it is the globins rather than the hemes of the two hemoglobins that are different. Let us propose that there is a surface region on the globin of the sickle cell anemia, hemoglobin molecule which is absent in the normal molecule and which has a configuration complementary to a different region of the surface of the hemoglobin molecule. This situation would be somewhat analogous to that which very probably exists in antigen-antibody reactions (*9*). The fact that sick-

[7] The patterns were very slightly different in that the known mixture contained 1 percent more of the sickle cell anemia component than did the sickle cell trait material.

[8] We are indebted to Dr. M. Moskowitz, of the Chemistry Department, University of California at Berkeley, for performing the ultracentrifuge experiments for us.

ing occurs only when the partial pressures of oxygen and carbon monoxide are low suggests that one of these sites is very near to the iron atom of one or more of the hemes, and that when the iron atom is combined with either one of these gases, the complementariness of the two structures is considerably diminished. Under the appropriate conditions, then, the sickle cell anemia hemoglobin molecules might be capable of interacting with one another at these sites sufficiently to cause at least a partial alignment of the molecules within the cell, resulting in the erythrocyte's becoming birefringent, and the cell membrane's being distorted to accommodate the now relatively rigid structures within its confines. The addition of oxygen or carbon monoxide to the cell might reverse these effects by disrupting some of the weak bonds between the hemoglobin molecules in favor of the bonds formed between gas molecules and iron atoms of the hemes.

Since all sicklemia erythrocytes behave more or less similarly, and all sickle at a sufficiently low oxygen pressure (*11*), it appears quite certain that normal hemoglobin and sickle cell anemia hemoglobin coexist within each sicklemia cell; otherwise there would be a mixture of normal and sickle cell anemia erythrocytes in sicklemia blood. We might expect that the normal hemoglobin molecules, lacking at least one type of complementary site present on the sickle cell anemia molecules, and so being incapable of entering into the chains or three-dimensional frameworks formed by the latter, would interfere with the alignment of these molecules within the sicklemia erythrocyte. Lower oxygen pressures, freeing more of the complementary sites near the hemes, might be required before sufficiently large aggregates of sickle cell anemia hemoglobin molecules could form to cause sickling of the erythrocytes.

This is in accord with the observations of Sherman (*11*), which were mentioned in the introduction, that a large proportion of erythrocytes in the venous circulation of persons with sickle cell anemia are sickled, but that very few have assumed the sickle forms in the venous circulation of individuals with sicklemia. Presumably, then, the sickled cells in the blood of persons with sickle cell anemia cause thromboses, and their increased fragility exposes them to the action of reticulo-endothelial cells which break them down, resulting in the anemia (*1*).

It appears, therefore, that while some of the details of this picture of the sickling process are as yet conjectural, the proposed mechanism is consistent with experimental observations at hand and offers a chemical and physical basis for many of them. Furthermore, if it is correct, it supplies a direct link between the existence of "defective" hemoglobin molecules and the pathological consequences of sickle cell disease.

3) *On the Genetics of Sickle Cell Disease*: A genetic basis for the capacity of erythrocytes to sickle was recognized early in the study of this disease (*4*). Taliaferro and Huck (*15*) suggested that a single dominant gene was involved, but the distinction between sicklemia and sickle cell anemia was not clearly understood at the time. The literature contains conflicting statements concerning the nature of the genetic mechanisms involved, but recently Neel (*8*) has reported an investigation which strongly indicates that the gene responsible for the sickling characteristic is in heterozygous condition in individuals with sicklemia, and homozygous in those with sickle cell anemia.

Our results had caused us to draw this inference before Neel's paper was published. The existence of normal hemoglobin and sickle cell anemia hemoglobin in roughly equal proportions in sicklemia hemoglobin preparations is obviously in complete accord with this hypothesis. In fact, if the mechanism proposed above to account for the sickling process is correct, we can identify the gene responsible for the sickling process with one of an alternative pair of alleles capable through some series of reactions of introducing the modification into the hemoglobin molecule that distinguishes sickle cell anemia hemoglobin from the normal protein.

The results of our investigation are compatible with a direct quantitative effect of this gene pair; in the chromosomes of a single nucleus of a normal adult somatic cell there is a complete absence of the sickle cell gene, while two doses of its allele are present; in the sicklemia somatic cell there exists one dose of each allele; and in the sickle cell anemia somatic cell there are two doses of the sickle cell gene, and a complete absence of its normal allele. Correspondingly, the erythrocytes of these individuals contain 100 percent normal hemoglobin, 40 percent sickle cell anemia hemoglobin and 60 percent normal hemoglobin, and 100 percent sickle cell anemia hemoglobin, respectively. This investigation reveals, therefore, a clear case of a change produced in a protein molecule by an allelic change in a single gene involved in synthesis.

The fact that sicklemia erythrocytes contain the two hemoglobins in the ratio 40 : 60 rather than 50 : 50 might be accounted for by a number of hypothetical schemes. For example, the two genes might compete for a common substrate in the synthesis of two different enzymes essential to the production of the two different hemoglobins. In this reaction, the sickle cell gene would be less efficient than its normal allele. Or, competition for a common substrate might occur at some later stage in the series of reactions leading to the synthesis of the two hemoglobins. Mechanisms of this sort are discussed in more elaborate detail by Stern (*13*).

The results obtained in the present study suggest that the erythrocytes of other hereditary hemolytic anemias be examined for the presence of abnormal hemoglobins. This we propose to do.

Based on a paper presented at the meeting of the National Academy of Sciences in Washington, D. C., in April, 1949, and at the meeting of the American Society of Biological Chemists in Detroit in April, 1949.

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The American Philosophical Society

Abstracts of Papers Presented at the 1949 Autumn Meeting, Philadelphia, Pennsylvania

Historical Botanical Collections of the American Philosophical Society and the Academy of Natural Sciences of Philadelphia

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Academy of Natural Sciences, Philadelphia

The Academy of Natural Sciences of Philadelphia is the depository of the largest series of early botanical collections preserved in the New World. These are plants gathered either in the latter half of the 18th or the first third of the 19th century, and they belong in equal degree to the Academy itself and to the American Philosophical Society, which deposited its collections with the Academy in 1897. Leading series are those from Francis Masson, who was in South Africa with Thunberg from 1786 to 1795 (ANSP); from Antoine Poiteau, in Haiti from 1796 to 1800 (ANSP); from Henry Muhlenberg, assembler from about 1780 to 1815 of the largest early herbarium in our country (APS); from Benjamin Smith Barton, at University of Pennsylvania from 1789 to his death in 1815, with collections made by John Wood in Australia in 1788, by Frederick Pursh in Virginia and elsewhere in 1806 and 1807, and by Meriwether Lewis on the Lewis and Clark Expedition from 1805 to 1807 (APS, but Pursh, Lewis, and other collections studied by Pursh in England are in part at ANSP); from Thomas Nuttall, over most of the United States and westward from 1807 to 1840 (ANSP, but earliest in APS); from William Baldwin in Georgia and Florida in 1815 to 181 (ANSP and APS); and from Lewis David von Schweinitz who was in the eastern United States from 1812 to 1834 and who made the earliest great American collection of Fungi (ANSP). These collections antedate by many years the building of great herbaria elsewhere in this country. One wonders why, after such a brilliant beginning, taxonomic investigation (other than that incidental to the amassing of personal herbaria) should have all but disappeared from Philadelphia by the middle of the 19th century.

Some Approaches to the Study of the Metal Requirements of Microorganisms

S. H. Hutner, ***Haskins Laboratories***

Knowledge of the inorganic requirements for life, conveniently studied in microorganisms, lags behind that of organic nutrition. The discovery that Vitamin B_{12} (the antipernicious anemia factor) contains cobalt has stimulated interest in the identification of the trace elements required for life and their role in metabolism. Such studies are limited or impracticable until deficiency states for these elements can be induced artificially. The case of Vitamin B_{12} illustrates these difficulties. The algal flagellate *Euglena gracilis* requires Vitamin B_{12}, and it is calculated that formation of one organism requires about 4,800 molecules of B_{12}, or 4,800 atoms of cobalt

on the assumption that the total cobalt required by the organism is contained in B_{12}, it is calculated that the known cobalt contamination (0.001%) of the iron added to the culture medium furnishes cobalt in at least a 3-fold excess. Iron itself is a relatively minor ingredient of the culture medium.

The hemoflagellate *Herpetomonas culicidarum*, which requires hemin as a growth factor, poses an analogous problem: Does the iron found in hemin represent its total iron requirement? As the iron requirement for aerobic forms is far greater than the cobalt requirement, it would seem good strategy to study first the hemin-iron relation in *Herpetomonas* before going on to the more difficult B_{12}-cobalt problem. The methods developed for solving these problems should prove applicable to identifying additional essential elements of the transitional series, should such elements be required.

The fundamental interest of such studies is underlined by the fact that vitamin B_{12} is concerned with the synthesis of desoxyribosenucleic acid, a substance bearing an intimate relation to the gene.

Varieties of Economic Law and Their Limiting Factors

John M. Clark, *Columbia University*

The material of economics, whether we like it or not, is largely indeterminate. Normal spending responds to changing income; but total normal spending (including capital outlay) may temporarily vary more than an income does, making income unstable and indeterminate. Bargaining units have much latitude, making prices and wages indeterminate, and bargaining practices evolve. Individual economic choices, besides being indeterminate, deal with ultimate and incommensurable human values.

Economists used to tell politicians what must happen under "economic law," despite political efforts to interfere. Now it seems more nearly true that politicians decide what is to happen, leaving economists speculating as to consequences, and whether or not actual policy is fatally unsound.

I have three suggestions for making analysis more nearly realistic. First, that we deal frankly with the specific content of human values—for example, health—instead of with abstract curves of choice in theoretical markets. Second, that wherever analysis deals with the kind of relations expressed in lines on graphs—for example, demand curves or cost curves—we turn the lines into zones or bands with width representing the degree of indeterminateness that exists. Then a maximum point or a point of intersection might become a wide range, within which the actual outcome would be decided by other factors than those expressed in the curves. This should enforce active study of these other factors, usually neglected. Third, that economics should frankly investigate what is sound, by standards which it should define—for example, "What should a wise government do about consumers' freedom of choice and why?" Any reasonable answer to this would by-pass existing theories built on refining inferences from utility curves or indifference curves.

This kind of analysis should produce, not fictitiously accurate results, but hypotheses calling for verification, thus bringing deductive thinking and inductive verification closer together.

Day Book of an Education: William Shippen's Student Days in London (1759–60) and His Subsequent Career

Betsy C. Corner, *Baltimore, Maryland*

Details about William Shippen, Jr.'s medical education had been entirely lacking until the discovery of a little diary he kept while a student in London (1759–60) was made unexpectedly some years ago by J. Hall Pleasants, of Baltimore, in whose safekeeping this 18th century manuscript has ever since remained. Through his courtesy and generosity, this little diary has recently become available for study.

In 1759 William Shippen, Jr. was enrolled at St. Thomas's Hospital, London, as a student in surgery. He also attended special lectures in midwifery, and he profited especially from intensive study of anatomy in the anatomical school established by John and William Hunter, two remarkable investigators of great importance in the history of medicine.

Shippen later studied in Edinburgh for a year and received his M.D. from the University of Edinburgh in 1761. He returned to Philadelphia in 1762 and almost immediately began to give a course in anatomy modeled upon the instruction he had received in London. To his credit, he transplanted from English to American soil not only the technical methods he had learned from the Hunters in their anatomical school, but the spirit of their experimental approach to the problems of human structure and of childbearing which were their special consideration.

Dr. Shippen's introductory lecture to his course on anatomy, given on November 11, 1762 at the State House in Philadelphia, marked the beginning of medical instruction of academic standard in the American colonies, and it set the pace for the future development of medical education in this country. Shippen also established the first classes for instruction in obstetrics, open to both sexes, in this country and started a small hospital for the lying-in care of poor women and the instruction of students in obstetrical procedure. That same year, 1765, he received his appointment as professor of anatomy and surgery in the medical department of the College of Philadelphia, the second medical professorship created by the trustees. His connection with the medical school was continuous until 1808 and for a longer time than that of any other member of the faculty of that period. Dr. Shippen is of special interest to the American Philosophical Society because he was elected to its membership in November 1767—182 years ago.

"Inanna's Descent to the Nether World": A New Tablet

Samuel Noah Kramer, ***University of Pennsylvania***

Some eight years ago, in the spring of 1941, the writer read before the American Philosophical Society the then available text of the Sumerian myth "Inanna's Descent to the Nether World." It had been pieced together in the course of several years from thirteen clay tablets and fragments located in the Istanbul Museum of the Ancient Orient and in the University Museum of the University of Pennsylvania. The poem, the writer then pointed out, was still unfortunately incomplete, and he concluded his reading with the hope that sooner or later some of the missing portions would be uncovered. The present paper reports the first and partial realization of this hope; a tablet belonging to the myth has turned up in the Yale Babylonian Collection at New Haven.

It contains 92 lines of text, and its latter half carries on the story of the goddess Inanna's resurrection and return to the earth from the point where the texts had previously broken off. Moreover this new material has a rather unexpected significance; it clears up a misconception concerning the Sumerian fertility god Dumuzi—the Biblical Tammuz—which has misled students of Mesopotamian religion for more than half a century. The tablet has been carefully copied by Ferris Stephens, curator of the Yale Babylonian Collection, and has been tentatively translated by the present writer. This paper and the resulting final publication will thus represent a cooperative effort of Yale University and the University of Pennsylvania.

The Physical Distinctions of Man

Adolph H. Schultz, ***Johns Hopkins University***

The physical distinctions of man are numerous and striking, if adult man is contrasted with other Primates at the completion of their growth. Early in life, man and other Primates are indistinct. Nearly all apparently fundamental, qualitative differences between adult man and apes are in reality of a mere quantitative nature, emerging gradually with age in consequence of diverging trends of development or of differing intensities in otherwise closely corresponding growth changes. The evolutionary modifications in the development of man include many accelerations as well as retardations which, though seemingly inconsequential, lead to marked peculiarities at the completion of growth.

The list of human distinctions is being constantly revised on the basis of accumulating information regarding the growth processes among Primates and can be newly evaluated by means of comparisons between the specific characters of man and those acquired by the manlike apes. In many bodily features man has remained conservative, not having changed as extremely as some other Primates. The most evident human distinctions are directly correlated with the early adaptations to the erect posture and the later enlargement of the brain. Other significant distinctions of man are connected with his latest evolutionary specializations: the prolongation in the main periods of life and the accompanying alterations in the duration and sequence of various developmental processes. Most characters, claimed as specific for man, do not represent radical innovations, but are merely the result of general evolutionary trends which have also produced very similar, though not as far-reaching, transformations in some or all of the anthropoid apes.

Characters which differ in man and the simian Primates in their average formation are often not truly distinctive, if individual conditions are taken into consideration, since the ranges of variations in many features are surprisingly extensive and can overlap in man and one or another of the anthropoids.

Generally speaking, man is clearly and constantly distinguished from all other Primates by his peculiar *combination* of characters which, singly, are often distinct only in regard to the degree of their specialization or the relative frequency of their occurrence.

These generalizations will be illustrated by discussions of examples of physical features distinctive of man.

The Chemical Combination of Insulin with Muscle and Its Hormonal Regulation

William C. Stadie, ***University of Pennsylvania***

Understanding of the fundamental problems of diabetes must await more complete knowledge than is available now of the chemical or physical mechanisms by which insulin affects the metabolism of mammalian tissues. The most prominent conception is that insulin affects one or more of the tissue enzymes which catalyze the multiple reactions concerned in the metabolism of fat, protein, or carbohydrate. Cell-free enzyme systems prepared from normal or pathological tissues should be ideal for the demonstration of such effects, but few demonstrations of insulin effects on such cell-free systems have been reported and these have been difficult to reproduce.

In sharp contrast, the effects of insulin upon intact cells, either *in vitro* or *in vivo*, have been unequivocally demonstrated by many workers. It is conceivable, then, that intact cellular morphology is a necessary condition for the action of the hormone. In that case, physical effects, such as on permeability, rather than chemical effects on enzymes, must be considered as possible effects of insulin. Irrespective of the precise mode of action, it is highly presumptive that, once having entered the cell, insulin must attach itself to some morphological element. This initial direct combination of insulin with the intact cell might indeed be the initial obligatory reaction required for metabolic activity of the hormone. We[1] have succeeded in demonstrating for the first time that such a chemical reaction occurs.

The demonstration is accomplished in the following

[1] The experimental data discussed here are taken from a paper by W. C. Stadie, N. Haugaard, J. B. Marsh, and A. G. Hills, *Amer. J. med. Sci.*, Sept., 1949.

way: a hemidiaphragm from a normal rat is equilibrated for one minute or less in a medium containing a small concentration of insulin. During this brief exposure to insulin firm combination has occurred with some constituent of the muscle cell, and the combined insulin alters the metabolic pattern of the muscle. This is shown by the fact that invariably the insulinized hemidiaphragm, when equilibrated in a glucose-containing medium, utilizes more glucose and synthesizes more glycogen than an appropriate control.

This phenomenon has been studied in the normal rat under a variety of conditions, and in rats with endocrine abnormalities, or those injected with various hormonal preparations. The results indicate that chemical combination of insulin with muscle is under hormonal control and appears to be an important factor in the regulatory mechanism of carbohydrate metabolism in the normal and diabetic animal.

The Development of Contractility

John S. Nicholas, ***Yale University***

The origin of contractility must naturally be a function of the developing embryonic tissue and in many organisms is initially common to many different kinds of tissue. In higher forms, however, the contractile nature of protoplasm is largely localized in muscle tissue, and the other tissues lose whatever contractile powers they may have possessed.

In the rat embryo, muscular movement is first noticed after 15 days of development and the flexions and rotations of the body of the fetus attain a considerable complexity before birth. During this period, as well as in the period before movement, the mesoderm has given rise through differentiation to muscle tissue markedly different from adult tissue, consisting of a loose arrangement free of fibers and with no discrete structures such as those normally described for muscle.

In the past few years there has been a tremendous advance in the study of the chemical nature of muscle and it was decided to investigate the chemical, physical, and morphological character of developing muscle, in order thereby to attain critical information which might be correlated with the contractile process.

The most pertinent studies are those which deal with the liberation of inorganic phosphate in chemically separated fractions of developing muscle at different stages of its differentiation, and the correlation of this activity with the nucleic acid content, the development of the muscle respiratory enzymes, and the appearance of birefringence in the developing muscle.

When embryonic muscle is fractionated, the activity of each fraction develops independently and at different rates. This general finding is similar to that of Shen and Boell on the development of the respiratory enzymes in developing muscle. Apparently definite contribution to the chemistry of contractile tissues is made independently by the separable substances. The development of birefringence pattern antedates the formation of actual contractile tissue by a considerable period—at least 12 hours—and occurs at least 24 hours before the first embryonic striations can be observed. The correlation of these and other factors throws considerable light upon the mechanism of beginning contraction.

Studies on the Development of the Cortex of the Brain

Louis B. Flexner, ***Carnegie Institution of Washington***

The aim of these investigations has been to establish how long before birth evidence of function can be found in the nerve cells of the cerebral cortex of the guinea pig and how functional development of these cells is related to their structural and chemical development. On microscopic examination, a series of abrupt changes was observed to occur in the nerve cells two-thirds of the way through gestation. Nerve processes start to grow rapidly, nucleoprotein appears in large quantity in the cytoplasm, and the nucleus ceases to increase in volume. These structural developments are accompanied by several changes. The enzyme, adenylpyrophosphatase, believed by its activity to yield useful energy to the cell, increases sharply in activity to the adult level. The activities of the respiratory enzymes, succinic dehydrogenase, and cytochrome C, responsible in part for the combustion of foodstuffs, also increase rapidly to the adult level. At the same period the nerve cells for the first time are electrically active; electrical potential changes can be recorded from the surface of the brain. These potential changes were demonstrated to arise from the cortex itself by local application of strychnine, which produces typical increase in electrical activity. The onset of this cortical electrical activity appears to be causally related to a sudden increase in the permeability of the nerve cells to sodium. This observation fits the hypothesis, advanced by others, that the potential change which follows the stimulation of peripheral nerve is due to the momentary appearance of a selective permeability to sodium.

It is concluded that the nerve cells of the cerebral cortex of the guinea pig begin to show functional activity about two-thirds of the way through gestation and that structural, chemical, and functional differentiation of these cells occurs at the same time or in rapid succession.

Redwoods—Occidental and Oriental

Ralph W. Chaney, ***University of California***[2]

The recently discovered redwood of Asia, *Metasequoia glyptostroboides,* shows relationship to the redwood of North America, *Sequoia sempervirens,* and to the swamp cypress, *Taxodium distichum.* Foliage and cones of the living trees provide characters by which they may be readily distinguished, but for nearly a century there has been confusion in the recognition of fossil specimens.

[2] Research associate, Carnegie Institution of Washington.

Large collections from the Cretaceous and Tertiary deposits of western North America have recently been restudied; criteria have been established for separating fossils of the three genera, and for setting up recognizable species.

During Cretaceous time, and at the beginning of the Tertiary period, *Metasequoia* was abundant at high northern latitudes. Its deciduous habit was well suited to a regime of summer rainfall and lowered winter temperature. With gradual reduction in rainfall and temperature. *Metasequoia* and its associates are recorded from successively more southerly stations, as far south as California; near the end of the Tertiary period it became extinct in North America, probably as a result of a shift to winter rainfall and summer drought. Its failure to survive in the southeastern United States along with *Taxodium*, which is also deciduous, cannot now be explained.

The geologic history of *Sequoia* has been somewhat different because of its evergreen habit. Never as abundant as *Metasequoia*, the ancestors of the California redwood were in past ages largely limited to foothills and to the borders of ancient seas. With the change in rainfall regime during later Tertiary time, *Sequoia* was restricted to the borders of the Pacific; in regions with summer fog it still survives. *Metasequoia* is known to exist only in the interior of China, where heavy summer rainfall is combined with moderate temperatures. Here in valleys so remote that they have not yet been completely deforested by land-hungry farmers, the redwood of Asia appears to be living the last chapter of its hundred-million-year existence.

TECHNICAL PAPERS

Mutual Interaction of Polyelectrolytes[1]

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Yale University, New Haven

Polyelectrolytes (*1*), even in the most dilute solution, give regions of high charge density in the neighborhood of each polyion, as a simple consequence of structure. We might therefore expect a strong electrostatic interaction between the fields of polycations and polyanions, which would lead to mutual precipitation. Results are herewith presented which verify this prediction.

In one series of experiments, portions of a dilute solution of sodium polyacrylate[3] were added to a solution of poly-4-vinyl-*n*-*N*-butylpyridonium bromide. Flocculent precipitates formed immediately; at concentrations as low as 10^{-5} normal in bromide ion (about 10^{-8} molar in polyelectrolyte), a distinct turbidity was still visible. The precipitation was followed quantitatively by measuring the turbidity of the mixtures with a Phoenix light-scattering photometer. Fig. 1 shows the results of a typical experiment in which successive portions of 10^{-3} normal polyacrylate solution were added to 40 ml of 25×10^{-6} normal polypyridonium bromide. Up to the equivalence point, the turbidity τ increased linearly with the amount of polyacrylate added; just beyond equivalence, the turbidity increase accelerated sharply, and after a ratio of

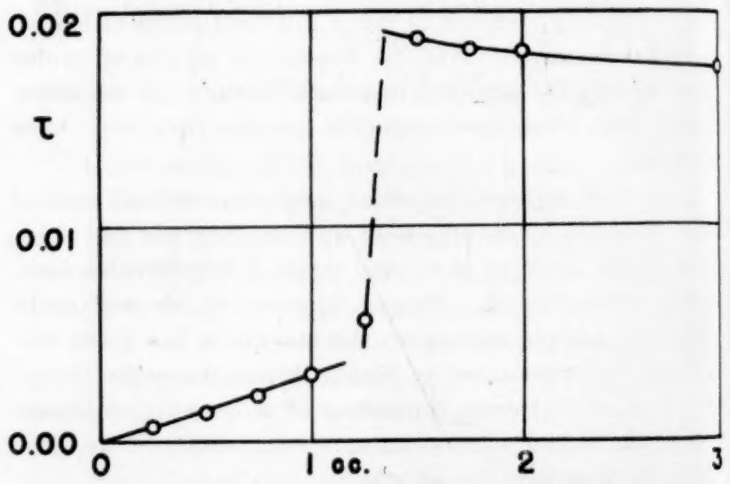

FIG. 1. Turbidimetric titration of polyvinyl-butyl-pyridonium bromide (40 cc of 25×10^{-6} normal) with sodium polyacrylate (10^{-3} normal).

about 1.5, slowly decreased with further addition of polyacrylate. Fig. 2 is a log-log plot of the turbidity obtained when 1.5 equivalents of polyacrylate were added to 1.0 equivalent of polypyridonium salt, the latter at varying stoichiometric bromide concentrations, *N*. It will be noted that the points fall on a 45° line, showing that the amount of precipitate is proportional to the concentration of the polypyridonium solution from which it was precipitated down to below millionth normal in bromide ion (approximately 10^{-9} molar in polybromide or 0.2 ppm by weight). The lowest concentration corresponds to the present limit of sensitivity of our optical equipment.

These results may be interpreted as follows: When polyacrylate is added to polypyridonium salt, the latter is initially in excess. Normally, each polyacrylate ion is surrounded by an atmosphere of sodium counter ions; when a polyacrylate ion-plus-ion cloud encounters polypyridonium ions, the strong attractive field between poly-

[1] Project NR 054–002 of the Office of Naval Research.

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[3] We are grateful to the Minnesota Mining and Manufacturing Company for a sample of polyacrylic acid and to the Dow Chemical Company for a sample of sodium polystyrene sulfonate.

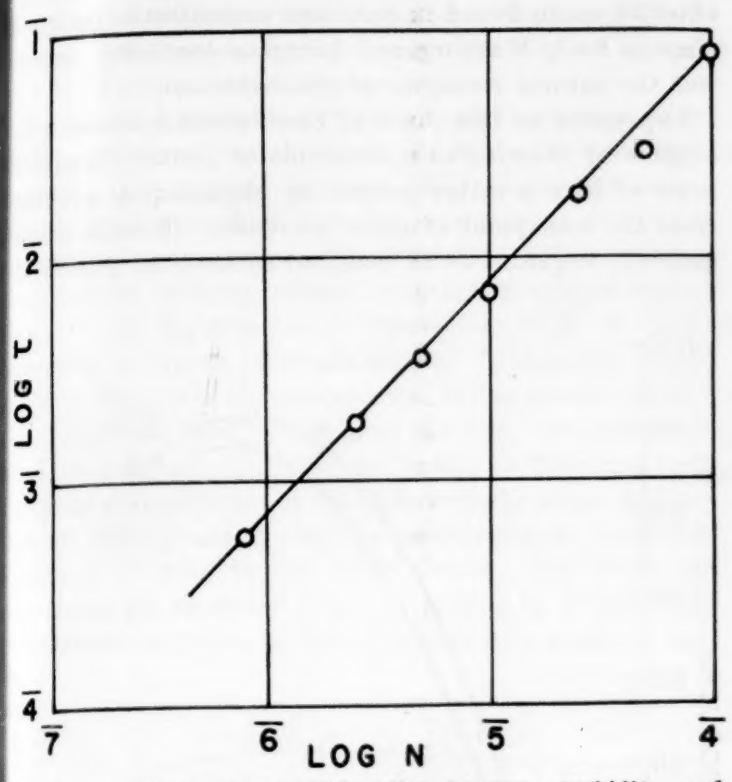

FIG. 2. Test of proportionality between turbidity and concentration of polyelectrolyte solution.

anion and polycations causes the polymeric ions to approach, and many of the monovalent gegen ions are displaced. The resulting aggregate is a compact insoluble cluster, cross-linked by electrostatic forces. We naturally do not expect perfect pairing of acrylate and pyridonium ions; those unpaired will be accompanied by their corresponding gegen ions. Since the pyridonium salt is in the medium into which the acrylate is poured and is initially in excess, the exterior of the cluster will be mostly polypyridonium chains. Then when excess acrylate is added, more polyanions attach themselves to the polycationic exterior of the precipitate particles, displacing both sodium and bromide counter ions until the excess charge is compensated. This hypothesis accounts for the sharp rise in turbidity just beyond the equivalence point followed by a leveling off at a fairly well-defined degree of excess. The subsequent slow decrease of turbidity probably is due to an electrostatic contraction of the particles of precipitate in the presence of excess ions, or else to coalescence of original particles. We plan to determine particle size.

In another series of experiments, solutions of sodium polystyrene sulfonate were mixed with polypyridonium bromide solutions. Again, flocculent precipitates formed. These experiments were carried out at higher concentrations of polyelectrolytes, so that conventional analytical methods could be used. The precipitates were centrifuged out, dried and weighed, and bromide ion in the supernatant liquid was determined by potentiometric titration. The results of a typical series of experiments are given in Table 1, where the polybromide solution (B) analyzed to 0.0180 normal in bromide ion. The concentration (approximately 0.005 normal in sulfonate) of the polysulfonate solution (S) was not accurately known, because the starting material contained some insoluble cross-linked products which were removed by centrifuging before the solution was used. (There also appeared to be some impurity of low molecular weight present.) In all the experiments of Table 1, polybromide was in excess. The first column gives the volume and sequence of reagents; e.g., "5 B: 20 S" means that 5 cc of polybromide was poured into 20 cc of polysulfonate. The second column gives the weight (in mg) of precipitate found, and the third, the mg of precipitate per unit volume of polysulfonate solution. The fourth column gives the number of milliequivalents of bromide ion used, and the fifth, the deficiency of bromide ion in the supernatant liquid after mixing with polysulfonate and centrifuging out the precipitate. This deficiency corresponds to bromide ions which accompanied the precipitate as neutralizing counter ions for the dangling polypyridonium chains.

TABLE 1

PRECIPITATION OF POLYVINYL-BUTYL-PYRIDONIUM BROMIDE WITH SODIUM POLYSTYRENE SULFONATE

Expt.	Ppt. in mg	mg/cc	Meq. Br^-	ΔBr^-
5 B : 20 S	47.9	2.40	0.090	0.0090
10 B : 20 S	40.1	2.00	0.180	0.0151
10 B : 10 S	21.6	2.16	0.180	0.0163
20 B : 10 S	21.2	2.12	0.360	0.0184
20 S : 5 B	37.0	1.85	0.090	0.0081
20 S : 10 B	25.9	1.30	0.180	0.0111
10 S : 10 B	14.9	1.49	0.180	0.0125
10 S : 20 B	16.8	1.68	0.360	0.0168

An average of 1.58 mg/cc precipitate was obtained when polysulfonate was poured into polypyridonium salt. Here the polycation was always in excess, so each polyanion formed the nucleus for a precipitate particle which contained more than a stoichiometric equivalent of polypyridonium, due to its dangling chain ends. When polybromide was poured into polysulfonate, however, an average of 2.17 mg/cc sulfonate solution was found. This result seems reasonable on the basis of our picture of the mechanism of precipitation; at first, polyanions were in excess, and each polycation added was the nucleus for a precipitate particle which contained dangling polyacrylate chains. Further addition of polycation added to the polyanionic chain ends, thereby increasing the weight of the precipitate which had formed initially. As seen in Table 1, the bromide ion deficiency was correspondingly greater for this sequence of mixing.

These precipitates are inherently different, both from familiar electrolytic precipitates like silver chloride and from the colloidal coagulates, such as those obtained from mixing suspensions of arsenious sulfide and of ferric hydroxide. In all three cases, electrostatic forces cause the approach of particles of opposite charge; with silver chloride, a substance of low intrinsic solubility is formed from silver ion and chloride ion, while with the colloidal precipitates, rigid, relatively massive particles coalesce. But with the polyelectrolytes, the individual charges are normal in their behavior; it is their high local concentration which brings about expulsion of gegen ions and solvent, and leads to precipitation. (Both sodium acetate and sodium benzene sulfonate, for example, will precipitate the polypyridine salt only at very high concentrations.)

When the charge density is as high as that corresponding to vinyl polymers such as we used (where an ionogenic group is attached to every second carbon atom of the chain), we would expect that any polycation will precipitate any polyanion. On the other hand, by preparing copolymers (*2*) with controlled spacing of charges, it might be possible to obtain polyelectrolytes which show a selective precipitability, according to whether opposite charges can be paired off geometrically or not. It might thus be possible to make a model similar to those postulated (*4*) for immunological reagents.

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Is Chloride a Coenzyme of Photosynthesis?

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One of the serious obstacles in the experimental study of the mechanism of photosynthesis has been the impossibility of separating the process from the activities of intact green cells. The recent work of Hill (*5*) makes it possible, however, to investigate outside the living cell the reaction most characteristic of photosynthesis in green leaves: photolysis of water resulting in the evolution of gaseous oxygen. The oxygen-liberating system resides in the chloroplasts, and remains functional when fragments or whole chloroplasts are removed from green leaves.

The photochemical evolution of oxygen by chloroplasts isolated from sugar beet and spinach was recently investigated by Warburg and Lüttgens (*6*), who reached the rather striking conclusion that chloride ion was a coenzyme essential for photochemical reactions in photosynthesis. That such a simple yet important fact had escaped the notice of all other workers in this field was indeed cause enough for Warburg and Lüttgens to remark how rash were all previous theories on the mechanism of photosynthesis. The evidence which led these authors to conclude that chloride is a coenzyme of photosynthesis was as follows. Isolated chloroplasts lose their capacity for oxygen evolution after several washings in water. They can be reactivated, however, by adding cytoplasmic fluid. The factor in cytoplasmic fluid responsible for reactivation of the chloroplast was found to be heat-stable. An analysis disclosed that cytoplasmic fluid contained chloride in 0.08 molar concentration. Addition of chloride alone as M/150 KCl brought about complete reactivation. Of the other anions tried, bromide was almost as effective, iodide and nitrate much less so, and fluoride, sulfate, thiocyanate, phosphate, and all the cations tried were without effect. Since chloride was the effective anion found in sufficient concentration in cytoplasmic fluid, Warburg and Lüttgens concluded that it was the natural coenzyme of photosynthesis.

Impressive as this chain of biochemical evidence is in support of chloride as a coenzyme of photosynthesis, it poses at once a rather perplexing physiological problem from the standpoint of plant nutrition. Chloride is not generally regarded as an essential element for growth of

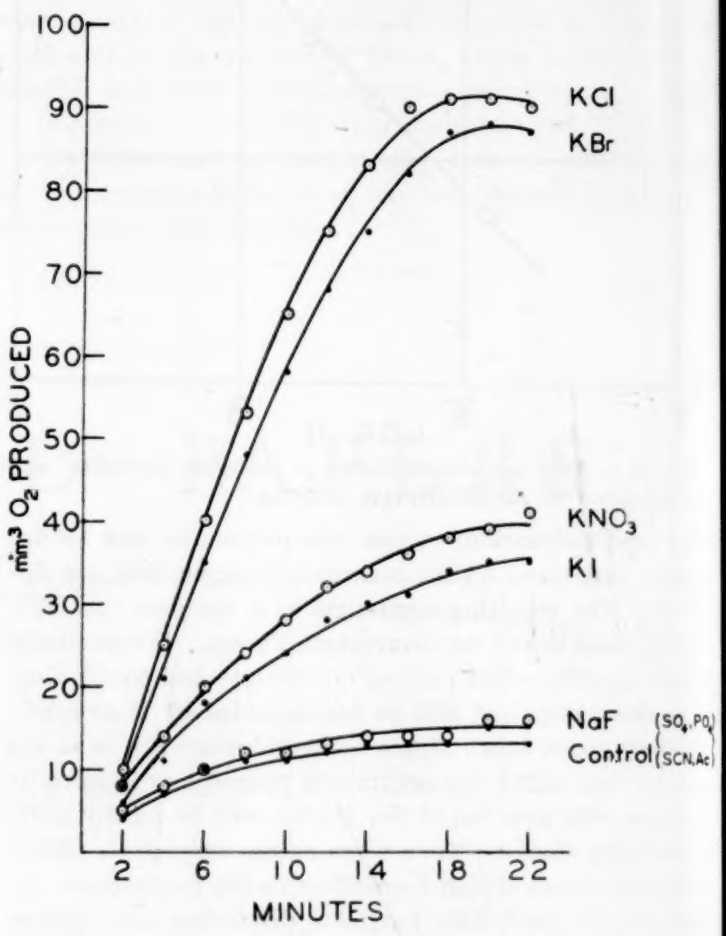

FIG. 1. Effect of anions (10^{-2} M) on oxygen evolution by illuminated chard chloroplasts. Reaction mixture: A chloroplast suspension containing 0.5 mg of chlorophyll, M/15 phosphate buffer, quinone as oxidant. Illumination at flask level approx. 28,000 lux, temp = 15° C. Other details of technique were similar to those previously described (*4*).

higher plants. Is it then possible that plants can get along in nutrient solutions without a coenzyme required for photosynthesis, a process indispensable for growth? The fact that Warburg and Lüttgens found appreciable amounts of chloride in their plants is not surprising. Chloride is widely distributed in soils and readily absorbed by most plants. Its presence in the plant, however, was hitherto regarded as incidental.

We undertook to investigate the problem by growing sugar beet and chard in nutrient solutions without chloride. Plants were grown in a nutrient solution supplemented with the micronutrients B, Mn, Cu, Zn, and Mo in amounts and from sources previously described (*1*), except that $MnSO_4$ was substituted for $MnCl_2$. As was expected, the plants made excellent growth in the nutrient solution to which no chloride was added. The chloroplasts from these plants were isolated (*2*) and

their oxygen evolution under the influence of light was measured manometrically, by a technique similar to that used by Warburg and Lüttgens (*4*).

Our results disclosed important areas of agreement with those of Warburg and Lüttgens, as well as several differences. An analysis of both chloroplasts and cytoplasmic fluid showed no chloride in either, as would be expected in plants grown without chloride. Chloroplasts, even without washing, showed only feeble oxygen evolution. In our experiments, in contrast to those of Warburg and Lüttgens, the addition of cytoplasmic fluid failed to reactivate the chloroplasts, but as already noted, our cytoplasmic fluid contained no chloride. On the other hand, we fully substantiated the finding of Warburg and Lüttgens that addition of chloride brought about activation of chloroplasts, giving us stoichiometric yields of oxygen in relation to the oxidant used. The effect of chloride on the course of oxygen evolution by illuminated chloroplasts is shown in Fig. 1, which also confirms the

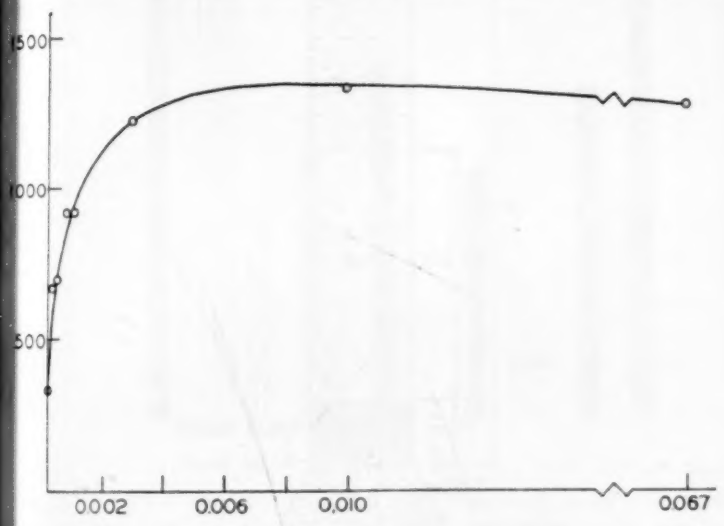

FIG. 2. Abscissas: KCl concentration. Ordinates: $Q_{O_2}^{chl}$. Effect of KCl concentration on rate of oxygen evolution by illuminated chloroplast fragments. $Q_{O_2}^{chl}$ = mm³ of oxygen/hr /mg of chlorophyll, computed from data obtained for the 6-min period from 1 to 7 min after turning on the light. Temp = 20° C. Conditions not specified were similar to those given in the legend for Fig. 1.

findings of these authors with regard to the influence of other anions on oxygen evolution. Bromide has an activating effect about equal to chloride; nitrate and iodide are much less effective; and sulfate, phosphate, thiocyanate, and acetate are without effect.

How should these results be interpreted? The intact plant is able to carry on normal photosynthesis without chloride, as judged by its excellent growth despite absence of this ion either in nutrient medium or in leaf tissue. Yet when chloroplasts are isolated from the same plant, they require chloride for vigorous progress of the photochemical reaction. One explanation would be that chloride acts in the leaf as a micronutrient, and that minute amounts of chloride which escape detection by usual chemical analysis may nevertheless be present in the nutrient medium as an impurity and reach the leaf. This explanation, although it cannot be ruled out entirely, is rendered unlikely by the data presented in Fig. 2. In this chart, the rate of oxygen evolution by illuminated chloroplasts ($Q_{O_2}^{chl}$) is plotted against chloride concentration. It will be seen that, whereas small additions of chloride brought about appreciable activation, a fairly high concentration, around 0.007 M, is required for full activation. This is in agreement with the value of M/150 KCl, reported by Warburg and Lüttgens as necessary for full activation in their experiments. Such relatively high concentrations of chloride are not uncommon in soil-grown plants, but there is strong evidence from these and numerous other experiments that plants can make excellent growth without the presence of measurable amounts of chloride either in the nutrient medium or in the plant. The other anion capable of giving full activation of

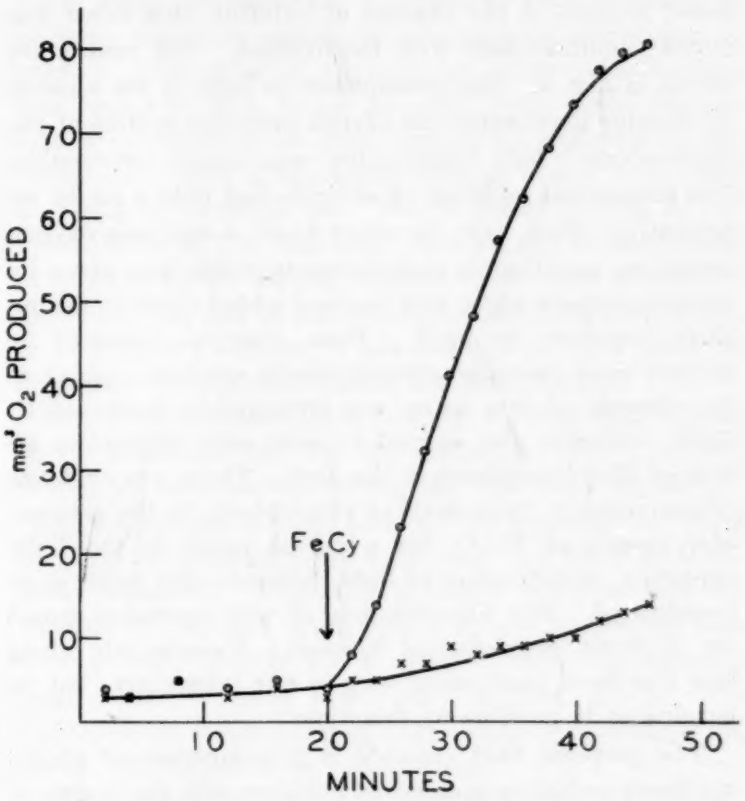

FIG. 3. Protective effect of chloride on illuminated sugar beet chloroplast fragments. *Circles*—illuminated for 20 min in the presence of chloride. At $t = 20$ min, the oxidant (ferricyanide) was tipped into the manometer vessel. *Crosses*—illuminated for 20 min in the absence of chloride. At $t = 20$ min, chloride and ferricyanide were added simultaneously. The concentration of chloride was 0.01 M KCl. A quantity of 1.5×10^{-7} moles of $K_3Fe(CN)_6$ was added to each vessel. Conditions not specified were the same as those given in legend for Fig. 1.

photochemical oxygen evolution, bromide, although readily absorbed and tolerated by plants in appreciable amounts, is not a common constituent of plants or soils, and there is even less reason for suspecting it as being essential for plant growth.

If the view that chloride or bromide is a coenzyme of photosynthesis *in vivo* is to be abandoned, how can the effect of these anions *in vitro* be explained? We have formulated the hypothesis that in the intact green cell photosynthesis goes on without participation of either chloride or bromide, but once the cell is broken there is a rapid light-induced deterioration of some cellular substance essential for the photochemical evolution of oxy-

gen by chloroplasts. Chloride or bromide is able to protect this substance against inactivation, but the intact cell accomplishes this in some other manner. This would explain the superfluousness of the halide *in vivo* as contrasted with its requirement *in vitro*.

The hypothesis was tested in the following manner. Isolated chloroplast fragments were illuminated without, however, adding the oxidant (in this case ferricyanide) which is necessary to bring about the evolution of oxygen. In one instance, chloride was added to the illuminated chloroplasts; the control contained no chloride. After 20 min of preexposure to light, the oxidant was added and the photochemical oxygen evolution was measured manometrically. To the chloroplast suspension which was exposed to light in the absence of chloride, this anion was added simultaneously with the oxidant. The results are shown in Fig. 3. The preexposure to light in the absence of chloride inactivated the oxygen evolution system of the chloroplasts. This inactivation was nearly irreversible. The subsequent addition of chloride had only a slight reactivating effect. On the other hand, a vigorous oxygen evolution, resulting in stoichiometric yields, was given by the chloroplasts which had received added chloride during their exposure to light. Thus chloride appeared to protect some essential photosynthetic substance which in the absence of this anion was irreversibly destroyed by light. Chloride also seemed to exert some protective action on the chloroplasts in the dark. There was evidence of inactivation from shaking chloroplasts in the manometer vessels at 15° C, for a period equal to the light exposure. Inactivation in light, however, was much more pronounced. The identification of this substance would be of great physiological interest. Experiments along this line have been under way in our laboratory, but no conclusion is possible at this time.

The proposal that chloride is a conenzyme of photosynthesis would have endowed chlorine with the status of an essential element for growth of higher plants. It would also have been the first instance in the history of plant nutrition where the essentiality of an inorganic element was established by the discovery of its biochemical function, in the absence of corroborative evidence from growth experiments according to specific criteria of indispensability (*3*). Our results, which speak against the role of chloride as a coenzyme in photosynthesis, also illustrate the contribution which growth experiments can make in evaluation of biochemical data bearing on the essential status of an inorganic element in nutrition of higher plants.

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The Action of Mineral-Ion Exchange Resins on Certain Milk Constituents

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With the discovery in 1936 by Adams and Holmes (*1*) that certain artificial resinous materials possess the ability to act as ion exchangers, new interest was aroused in this field. A large number of such synthetic ion-exchange

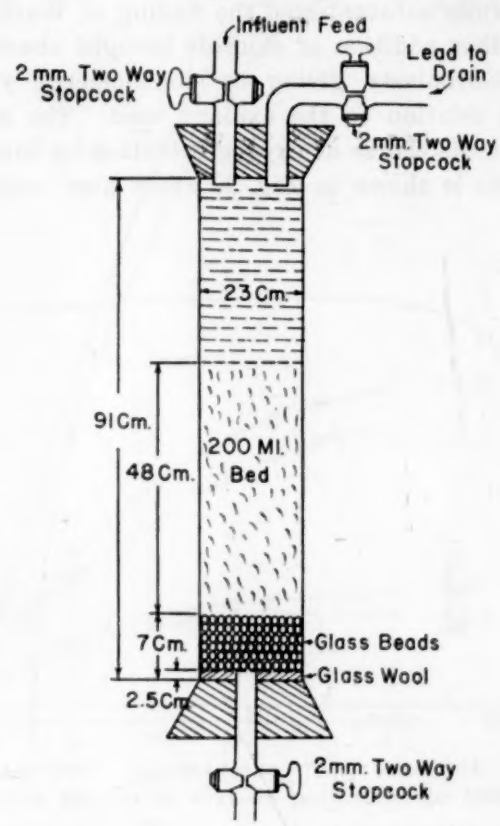

FIG. 1. Ion-exchange column. The Zeo-Karb-H column was initially conditioned with 400 ml of 5% NaCl, downflow at a rate of 13.7 ml/min, backwashed at flow rate to give 50% bed expansion for 5 min, regenerated with 450 ml of 0.407 *N* HCl at 17 ml/min, and washed with distilled H_2O at the same rate until free of acid. The De-Acidite column was exhausted with 2,000 ml of 0.100 *N* HCl, backwashed, regenerated with 280 ml of 0.75 *N* Na_2CO_3 at 4.5 ml/min, and washed free of alkali.

materials are now commercially available, and various laboratories have been experimenting with their properties when used to treat milk. It was felt that some fundamental studies should be made also on the action of typical anion-exchanger and cation-exchanger resins with simple solutions of the known major inorganic milk constituents, at concentrations as they normally occur.

In 1933, Lyman and co-workers (*5*) discovered that by the action of certain natural base-exchange materials called zeolites, the mineral constituents of milk could be modified, chiefly by decrease in calcium ion, so as to im-

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prove the ease of digestion and assimilation of milk through the softer curd obtained. Other investigators (*4*) have found that M.I.E. (mineral-ion exchange)-treated milk added to evaporated milk was capable of stabilizing the product against coagulation during sterilization at 240° F for 15 min. The M.I.E. milk can be used as a fluid, powder, or concentrate. Burgess[2] has a patent that covers the use of an artificial ion-exchange material for removing calcium ions more or less completely from milk. Otting (*6*) has discussed the problems which were surmounted in the application of the

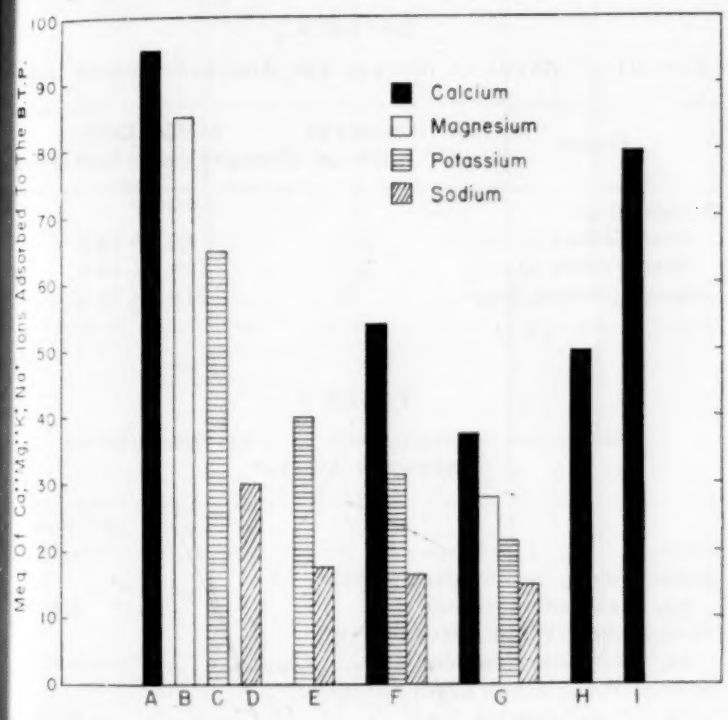

FIG. 2. Adsorption of cations to the B.T.P. by Zeo-Karb-H. Two thousand ml of the following solutions was passed through the column. *A*. $CaCl_2$, 100 meq./l; *B*. $MgCl_2$, 100 meq./l; *C*. KCl, 100 meq./l; *D*. NaCl, 100 meq./l; *E*. Binary soln. of $CaCl_2$ and NaCl, 50 meq. each/l; *F*. Ternary soln. of $CaCl_2$, KCl, and NaCl, 33.3 meq. each/l; *G*. Quaternary soln. of $CaCl_2$, $MgCl_2$, KCl, and NaCl, 25 meq. each /l; *H*. Binary soln. of $CaCl_2$ and HCl, 50 meq. each /l; *I*. Binary soln. of $CaCl_2$ and citric acid, 50 meq. each /l. Influent flow rate 50 ml/min; column regenerated with 450 ml of 0.407 *N* HCl at 17 ml/min, followed with 200 ml distilled H_2O at the same rate, then a fast rinse at 50 ml/min for 10 min.

mineral-ion exchange principle in milk products, and the equipment and procedure to be used.

Numerous other investigations have been made on the use of ion-exchange substances in the separation of cations from anions (*7–10*), in the separation of amino acids, purine, and pyrimidine bases, alkaloids, and many other substances (*2, 3, 11*).

The present paper describes briefly the action of a representative pair of commercial anion and cation exchange resins on the various salts known to occur in milk. The salts were prepared in water solutions containing one, two, three, or four of the cation or anion constituents found in milk, in equivalent concentrations, and then given appropriate ion-exchanger treatment.

[2] Burgess Zeolite Co. Ltd. British patent, No. 542,846 (1942). C. A. 36: 4217–4.

Zeo-Karb-H and De-Acidite[3] were the exchangers employed. The columns were set up as shown in Fig. 1. The air-dry exchanger was added to the column until a 200-ml backwashed and drained volume was obtained. Before using the columns in experimental procedures, each was put through two cycles of exhaustion and regeneration in order to condition the beds.

The break-through point (B.T.P.) was designated as that point at which the concentration of a given ion in the effluent reached a value which was 5% of the initial concentration, while 95% was still being adsorbed by the exchanger. The results were expressed in terms of milliequivalents adsorbed to the B.T.P.

The increasing order of removal for the cations studied, either from individual ion solutions or from mixtures of all four, was from sodium to potassium to magnesium to calcium. The type of opposite ion present in solution was found to be a factor in some cases, since calcium ions were found to be more completely removed by the cation exchanger if the anion present was citrate, than if it was the chloride ion. This is shown in Fig. 2.

When the B.T.P. has been reached, the concentration of the ion in the effluent rapidly approaches the initial concentration, and in the solutions containing two, three, or four of the cations, it has been found that some of them may surpass their initial ion concentration.

The cations present in a complex solution that appear to be least adsorbed by the exchanger were found in reality to be removed during the first part of the exchange run, then released later by the regeneration effect of the other cations in the solution which were preferentially adsorbed. Thus, the hydrogen ion from the exchanger in the acid cycle is not always exchanged each time a cation enters the exchanger, since the entering cation may replace a previously adsorbed cation. The hydrogen ion-cation exchange for solutions containing one cation was found to be quantitative.

When single, binary, and ternary solutions of hydrochloric, citric, and phosphoric acid were passed through a bed of an anion exchanger, De-Acidite, it was found that the order of removal for acids in mixtures was different from the order in trials with solutions containing but one acid. Thus, the order of removal of the acids from individual solutions increased from hydrochloric to citric to phosphoric, but in a solution containing all three acids the order of increased removal was from citric to phosphoric to hydrochloric.

A 0.6% solution of urea, creatine, and creatinine, each being present in 0.20% concentration, was subjected to cation-exchange treatment. A 100% removal of these substances was effected from the first 400 ml of effluent. A 50% removal still occurred after 1600 ml of solution had passed through the bed. These substances were not adsorbed from solution by De-Acidite, an anion-exchange resin.

From the experimental results obtained in this study on less complicated true solutions, and from unpublished data on the action of ion-exchange resins on skim milk, whole milk, and deproteinized milk, ion-exchange ma-

[3] Manufactured by the Permutit Company, New York City.

terials appear to offer a variety of possibilities for modifying the mineral components of milk, either by removal of certain ions, by substituting other ions for normal ions present, or by both operations. Unpublished work, by one of the authors, E. F. Almy, on the calcium removal from cation-exchanged skim milk, shows that removals for calcium start at 83% and drop to 39% as the exchange run progresses, whereas the adsorption for phosphorus remains constant at approximately 7–8%.

Applications of ion-exchange milk to produce smoother ice cream, to improve the quality of baked goods made with milk, and to improve various other dairy products have been suggested, but await further investigation. A local company[4] has patent applications filed covering complete demineralization of cheese whey for the production of lactose with low mineral (ash) content, and for the production of the M.I.E. milk previously mentioned. It also has filed patent applications covering the production of a powdered cream which has been rendered heat-stable by ion exchange to lower the calcium ion content before drying.

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The ΔG/ΔP Ratio after the Administration of Dextrose as an Index of Insular Function

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In the course of some investigations on the decrease in serum inorganic phosphorus produced by the administration of glucose, it was found useful to relate increase in blood sugar values to decrease in inorganic phosphate.

It is well known that deficiency in insulin production is accompanied by high blood sugar levels as determined a half hour after administration of the carbohydrate, and vice versa, that increased insulin secretion leads to low values. However, there are factors, such as rate of glycogenolysis, secretion of epinephrine, storage ability of the tissues, and different rates of intestinal absorption, that may easily alter blood sugar levels.

The decrease in serum inorganic phosphorus which follows dextrose administration (*1, 5, 7*) depends upon the liberation of insulin; in fact, it does not take place in the pancreatectomized animal (*2*). Epinephrine also produces a similar decrease, but only in animals with intact pancreas (*8*). Insulin and epinephrine produce the same changes in inorganic phosphorus, insulin acting by itself (*9*), and epinephrine through the discharge of insulin induced by the elevation of blood sugar. The decrease in inorganic phosphate determined by glucose is not affected by liver deficiency (*2*).

These facts show the importance of studying systematically changes in serum inorganic phosphorus during the glucose tolerance test, in metabolic clinics, and in research laboratories. The results of such investigations are herewith reported.

The work was carried out in apparently normal dogs and in alloxan-treated dogs. Some of the normal dogs were in poor nutritional condition. A fasting sample was taken from the saphena vein, and immediately 1 ml/kg of body weight of a 50% glucose solution injected into this vessel; 30 min later a second sample was taken and the injection of dextrose repeated; 30 min later the last sample was drawn. The blood sugar determinations were carried out in duplicate, according to the Nelson method (*6*) and the serum inorganic phosphate determinations were also done in duplicate by the Fiske-Subbarow procedure (*4*). The ΔG/ΔP ratio was calculated in the following manner: the difference between the blood sugar value at 30 min and the initial value was divided by the difference between the initial serum phosphate and its value at 30 min. Results are shown in Tables 1 and 2 and Fig. 1.

From these results some conclusions can be established.

TABLE 1

The ΔG/ΔP Ratio, in Normal and Alloxan-treated Dogs

Group	Number of animals	ΔG/ΔP Ratio averages ± standard errors
Normal dogs		
Staub effect present	26	60.4 ± 18.2
Staub effect absent	17	105.9 ± 53.5
Alloxan-treated dogs	9	316.8 ± 75.2

TABLE 2

Statistical Significance of the Differences between Means*

	p Values
Normal dogs, Staub effect present, vs. Staub effect absent	0.40
Normal dogs, Staub effect present, vs. alloxan-treated dogs	< 0.01
Normal dogs, Staub effect absent, vs. alloxan-treated dogs	0.02

* Student's test (*3*).

[1] With the technical assistance of Miss Jeanne Lopez, Miss Luisa Maria Andueza, and the cooperation of Dr. Alfonzo Podrizki.

[4] M. & R. Dietetic Laboratories, Columbus, Ohio.

The ratio $\Delta G/\Delta P$ is a useful indicator of insular function, being less affected than the Staub effect by malnutrition and other factors that impair carbohydrate storage or utilization. While 39.5% of the apparently normal dogs did not have the Staub effect, only 16.2% of the animals had a $\Delta G/\Delta P$ ratio smaller than –100, the

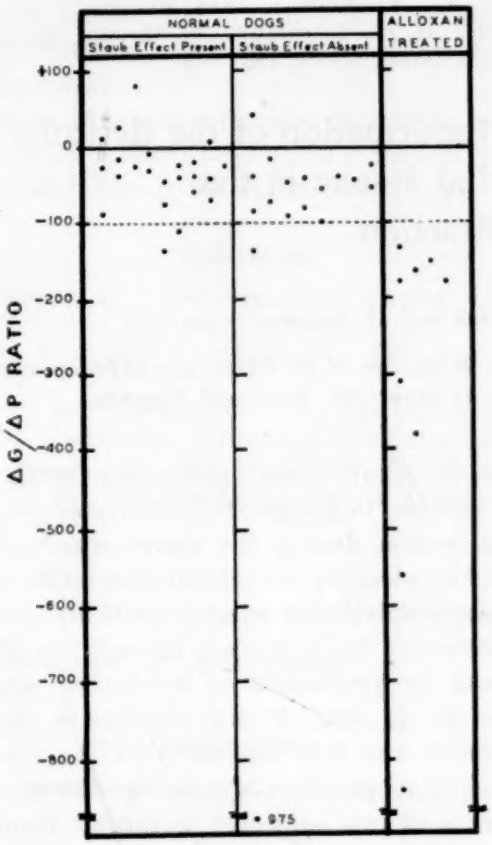

FIG. 1.

assumed normal limit. All of the diabetic dogs had a ratio smaller than –100.

The positive values shown had a negative glucose difference, suggesting a faster rate of sugar removal from the blood. When the index is positive, because of a positive phosphorus difference, it means there is a defective production of insulin.

The results in diabetic patients and in normal human subjects will be reported elsewhere; they are in agreement with the experimental data obtained.

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Amino Acids in the Mitochondrial Fractions of Tissues as Determined by Paper Partition Chromatography

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The biochemical properties of intracellular structures identified as mitochondria have received considerable attention recently. However, little is known of the nature of the protein moiety of these important cell constituents. As a first step in the characterization of mitochondrial protein, the amino acids in acid hydrolyzates were studied by two-dimensional paper chromatography (*1*, *2*).

The method used for the isolation of the mitochondria was essentially that of Hogeboon *et al.* (*3*) with the exception that additional cycles of low and high speed centrifugation were employed to insure the attainment of maximal uniformity of the sedimented material. The isolated particles were the same size and shape as the structures identified as mitochondria in smears made from homogenates, and in free cells found in the sediment from the first low speed centrifugation. These particles possessed the same staining characteristics, with Janus green B before fixation, and with aniline-acid fuchsin after fixation with osmic acid, as do mitochondria in cells. The isolated mitochondria were hydrolyzed with 6 *N* HCl in sealed tubes for 24 hr and aliquots were subjected to chromatography. Samples were treated with H_2O_2 to enable detection of cystine as cysteic acid and methionine as methionine sulfone. Numerous chromatograms were

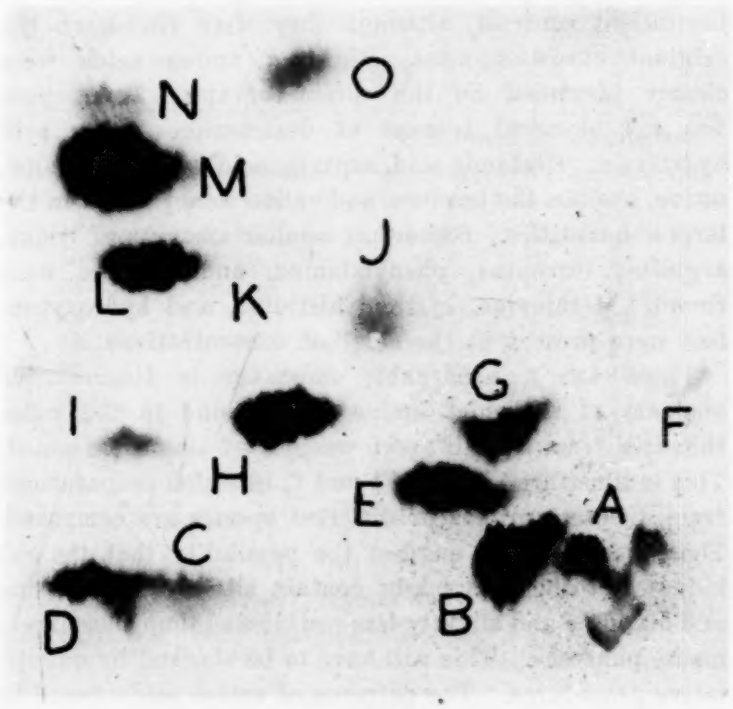

FIG. 1. Photograph of chromatogram from hydrolyzate of rat kidney mitochondria. (A) Aspartic acid, (B) glutamic acid, (C) lysine, (D) arginine, (E) glycine, (F) cystine (cysteic acid), (G) serine, (H) alanine, (I) histidine, (J) threonine, (K) methionine (methionine sulfone), (L) valine, (M) leucine and isoleucine, (N) phenylalanine, (O) tyrosine.

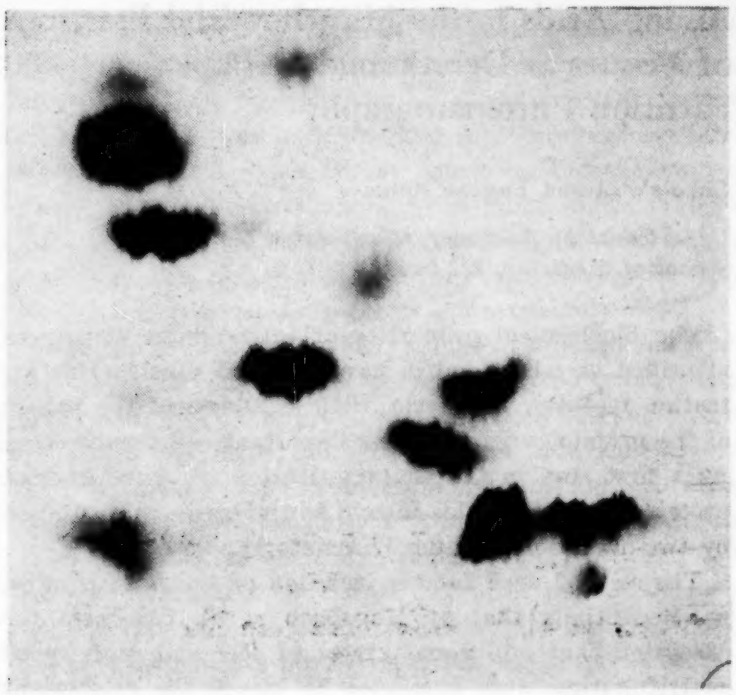

FIG. 2. Photograph of chromatogram from hydrolyzate of mouse pancreas mitochondria.

run on each sample, and comparisons between samples were made for chromatograms on which the amino acids occurring in the highest concentrations gave spots of approximately equal area and intensity, as shown in Figs. 1 and 2. Mitochondrial fractions of the liver and kidney of the mouse and rat, and of pancreas, mammary gland, hepatoma, squamous cell, and mammary carcinomas in the mouse were analyzed.

Photographs of two typical chromatograms are shown in Figs. 1 and 2. The visible constituents are identified on Fig. 1. The yellow spots given by proline and hydroxyproline are not visible on the photographs because of insufficient contrast, although they were visible on the original chromatograms. Eighteen amino acids were clearly identified on the chromatograms. Tryptophan was not observed because of destruction during acid hydrolysis. Glutamic acid, aspartic acid, glycine, alanine, serine, proline, the leucines, and valine were present in the largest quantities. Somewhat smaller amounts of lysine, arginine, threonine, phenylalanine, and tyrosine were found. Methionine, cystine, histidine, and hydroxyproline were present in the smallest concentrations.

There was a remarkable constancy in the relative amounts of the chief amino acids found in the mitochondria from the different samples of tissue examined. This is illustrated in Figs. 1 and 2, in which preparations from different organs of different species are compared. The chromatograms suggest the possibility that the rat kidney mitochondria might contain slightly more lysine and histidine and slightly less methionine than those from mouse pancreas. This will have to be checked by quantitative procedures. The patterns of amino acids found in the preparations from the malignant tissues examined were virtually identical with those obtained from normal mouse tissues.

The similarity of the amino acid patterns found in the mitochondrial fractions of the various tissues studied suggests that there is a characteristic protein, or combination of proteins, associated with these particles. The results also indicate that the quantity of the dicarboxylic amino acids exceeds that of the basic amino acids.

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On the Participation of the Reticulo-endothelial System in the Alarm Reaction

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We wish to report upon experiments which revealed a marked increas in phagocytic activity of the reticulo-endothelial system during the alarm reaction, that is, during the first phase of the general adaptation syndrome.

The reticulo-endothelial system probably participates in the defense of the organism through its phagocytic activity, and by production of antibodies, agglutinins, antitoxins, etc. (*1, 4*). A local increase in the activity of this system has been described in the thymus (*5*) after exposure to various acute nonspecific stresses, and in the spleens of rats subjected to chronic inanition (*3*) or injected with cortical extracts (*2*). Stimulated by these findings, we have performed two series of experiments in order to clarify the relationship between the reticulo-endothelial system and the hormonal and metabolic changes which occur during the alarm reaction.

In our first experiment, 44 piebald male rats (average body weight 150 g) were divided into four equal groups: group I served as untreated controls; groups II, III, and IV were fasted 48 hr, and during the last 24 hr were submitted to various stresses, such as cold (0–5° C), spinal cord transection (at the height of the 7th cervical vertebra), and repeated, exhaustive, forced exercise. All animals were injected intravenously with 2 ml of a dilute solution of Higgins India ink (1 part India ink to 5 parts physiologic NaCl solution) 1 hr before they were killed. At autopsy, naked eye inspection showed, in all the stressed animals, a markedly increased deposition of India ink in the lung, kidneys, adrenals, bone marrow, and the "hibernating gland." In the hibernating gland, this was accompanied by an acute discharge of lipid granules, hyperemia, and edema. These changes are characteristic of the alarm reaction and have been given special attention elsewhere (*6*). Compared to the controls, the India ink deposition in the liver of the alarmed rats did not seem to be significantly increased, while in the spleen there was a diminution of India ink deposition.

Subsequent histological examination confirmed and extended the autopsy findings. There was increased phagocytosis in the lung, kidneys, adrenals, bone marrow, hiber-

nating gland, thymus, and lymph nodes. Phagocytosis was not increased in the hepatic macrophages of the alarmed animals. In the spleen, which undergoes marked atrophy during the alarm reaction, there was a slight decrease in the India ink phagocytosis.

In a second series of experiments, 20 piebald male rats (average body weight 130 g) were divided into four groups and treated as in the first experiment. The autopsy and histologic findings confirmed the observations already described.

The results of our experiments suggest an active participation of the reticulo-endothelial system in the defense of the organism during the alarm reaction.

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Colorimetric Estimation of Noradrenalin in the Presence of Adrenalin

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Since noradrenalin generally occurs in animal tissues in a variable mixture with adrenalin (*1*, *3*, *5*), it has become desirable to find methods for quantitative assay of both substances in a mixture. This can be done by taking advantage of the difference in activity ratio of noradrenalin and adrenalin on various test objects, such as the blood pressure of the cat in chloralose anesthesia, and the hen's rectal cecum (*3*, *5*). Although the general type of effect is the same, noradrenalin may have 2–4 times the effect of adrenalin on the cat's blood pressure weight for weight, but only 1/10–1/50 of the activity of adrenalin on the fowl's rectal cecum. By computing the results of the assay, the relative amounts of noradrenalin and adrenalin may be estimated with a fairly high degree of accuracy. Though the biological method has the advantage of high specificity and requires only small amounts of active material, it is time-consuming and necessitates the use of at least two test animals. For this reason a simpler chemical method has been developed. The procedure given herein has been adopted as satisfactory.

The biological material is purified either by adsorption on alumina, according to the method of Shaw, modified by von Euler (*4*), or by the method of Bergström and Hansson (*2*), based on the ion-exchange principle.

The colorimetric method is based on the formation of noradrenochrome and adrenochrome on oxidation with iodine. The adrenochrome formation is complete when iodine is allowed to act for 1½ min at pH 4.0, whereas only about 10% of the noradrenalin is transformed into noradrenochrome under the same conditions. On 3-min treatment with iodine at pH 6.0, maximal formation of noradrenochrome and adrenochrome is attained.

The procedure is as follows: To an amount of purified extract containing 20–200 μg catechol derivatives, 1 ml *n*-acetate buffer of pH 4 and 0.2 ml of 0.1 *N* iodine solution is added. After precisely 1½ min, excess iodine is removed with 0.05 *N* sodium thiosulfate. The color is read within 5 min against a blank without iodine in a photometer at wavelength 529 mμ. The procedure is repeated with a second sample using acetate buffer pH 6 and 3-min iodine treatment. Standard readings are made with 100 μg adrenalin and noradrenalin at pH 4 (1½ min), and pH 6 (3 min), giving the calibration factors for both substances and the percentage of noradrenalin oxidized at pH 4 in 1½ min. At 529 mμ, the adrenochrome figure is the same on oxidation at pH 4 and pH 6.

Computation of results:

a = reading at pH 4 (1½-min iodine treatment)

b = " " pH 6 (3- " " ")

m = calibration factor for adrenalin $\left(\frac{100}{\text{reading for } 100\ \mu g}\right)$

n = " " " noradrenalin "

p = relative amount of noradrenalin oxidized at pH 4 in 1½ min

$$\begin{cases} \text{noradrenalin} = n \cdot \dfrac{b-a}{1-p} \\ \text{adrenalin} = m \left[a - p \dfrac{(b-a)}{1-p} \right] \end{cases}$$

With mixtures of noradrenalin and adrenalin in varying proportions, the results indicated in Table 1 were obtained.

TABLE 1

Amounts added:		Calculated from readings:	
adrenalin	noradrenalin	adrenalin	noradrenalin
μg	μg	μg	μg
10	90	11.8	86.8
20	80	20.5	80.5
30	70	30.3	68.6
40	60	40.8	59.5
50	50	50.7	47.7
60	40	61.1	39.5
70	30	70.0	30.4
80	20	79.7	19.5
90	10	90.8	9.6

The method has been repeatedly tested on purified suprarenal extracts and results have agreed well with those obtained from biological methods.

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Syntheses of Isotopically Labeled 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Derivatives

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The great interest in and wide use of 2,4-dichlorophenoxyacetic acid (2,4-D) in recent years has prompted us to investigate the synthesis of this compound and some of its derivatives incorporating radioactive isotopes.

One synthesis employs radioactive I^{131} and leads to 2,4-dichloro-5-iodophenoxyacetic acid-I^{131} through the sequence of the reactions shown in Fig. 1.

The 5-amino compound was prepared as indicated (*3*), diazotized and treated with radioactive sodium iodide, diluted with inactive material, thus yielding iodine-labeled 2,4-D. The butyl ester was obtained from the free acid through the acid chloride prepared by means of thionyl chloride (*3*). In this manner we have synthesized 75 mg of the free acid, 80 mg of its morpholine salt, and 92 mg of its butyl ester, showing specific activities of 28, 23, and 24 μc/mg, respectively.

We have also developed a method leading to 2,4-dichlorophenoxyacetic acid-1-C-14 by means of the sequence in Fig. 2.

Potassium acetate-1-C-14 prepared in the conventional manner (*1*) is chlorinated in nitrobenzene, using a modification of Ostwald's synthesis (*2*). Alkaline condensation of the chloroacetic acid, which is never isolated as such, with 2,4-dichlorophenol then leads to the desired product. In this manner, 0.68 g of C^{14} labeled 2,4-D was obtained, and showed a specific activity of 1.0 μc/mg. Complete details of the work will be published elsewhere.

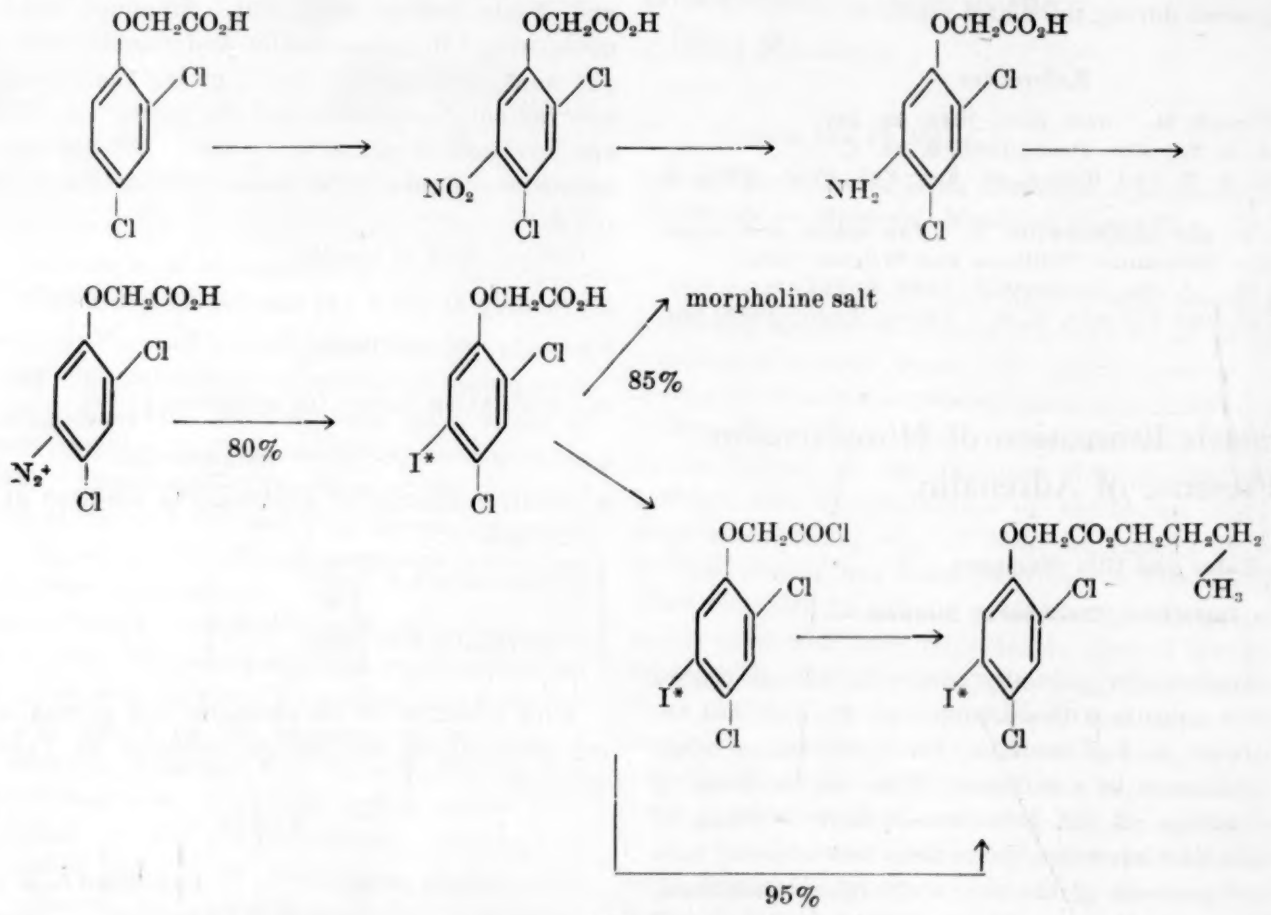

FIG. 1.

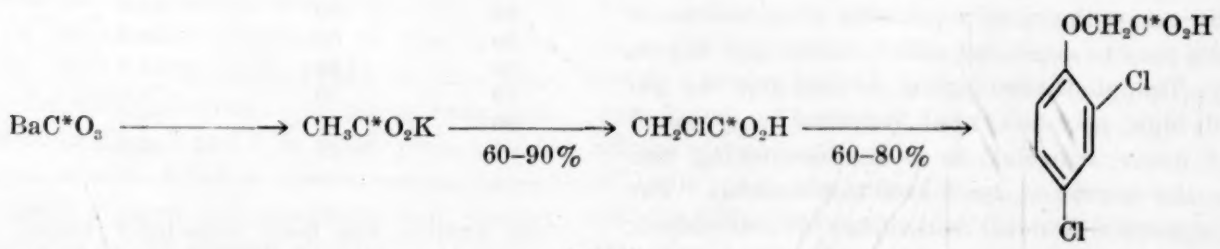

FIG. 2.

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Synergism of Thromboplastic Extracts: Synergistic Activity of Rabbit Lung and Brain Thromboplastic Extracts

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In the course of attempts to standardize thromboplastic materials, an apparent synergism of some of these materials was encountered. The purpose of this paper is to present evidence of such an effect in the thromboplastic activities of rabbit lung and rabbit brain mixtures.

Acetone-dried rabbit brain and acetone- or air-dried rabbit lung were extracted with 0.85% NaCl and tested on frozen plasma from normal human subjects, utilizing our modified one-stage technique of Quick. Results of volumetric mixtures of the extracts when tested in this way are shown in Table 1 and graphically in Fig. 1, curves A, B, and C.

The data demonstrate that there is increased thromboplastic activity of mixtures of brain and lung extracts above that which would be anticipated by the process of mixing. This is particularly well demonstrated by curve B, where an extremely "slow" lung preparation made by acetone drying was mixed with a "rapid" brain

TABLE 1

Brain-lung ratio	PLASMA A* Observed clotting time (C.T.) in sec	PLASMA A* Calc. add. C.T. in sec	PLASMA B† Observed C.T. in sec	PLASMA B† Calc. add. C.T. in sec	PLASMA C* Observed C.T. in sec	PLASMA C* Calc. add. C.T. in sec
100B 0L	14.8		14.7		21.0	
90B 10L	—	—	14.5	17.3	17.0	20.4
80B 20L	13.2	14.8	13.8	19.8	16.7	19.8
70B 30L	12.9	14.8	13.1	22.3	15.6	19.2
60B 40L	12.8	14.8	11.9	24.8	14.0	18.6
50B 50L	12.3	14.9	12.6	27.3	15.0	18.0
40B 60L	12.2	14.9	12.7	29.8	13.7	17.4
30B 70L	13.4	14.9	13.0	32.3	14.2	16.8
20B 80L	14.1	14.9	13.8	34.8	13.7	16.2
10B 90L	14.0	14.9	15.5	37.3	13.5	15.7
0B 100L	14.9		40.4		15.1	

* Clotting time obtained with air-dried lung thromboplastin.

† Clotting time obtained with acetone-dried lung thromboplastin.

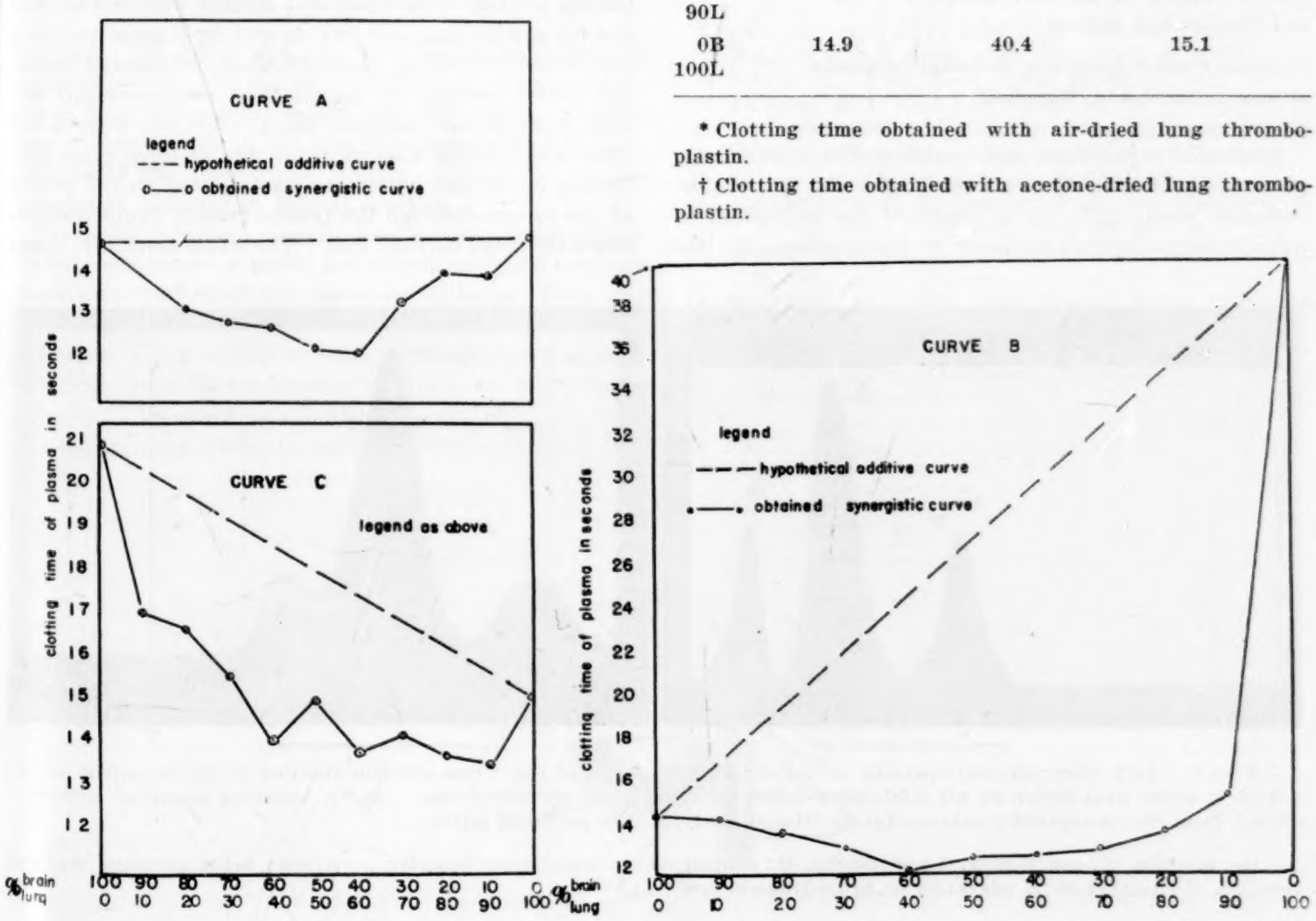

FIG. 1.

preparation. A mixture of one part of brain extract with nine parts of lung extract exhibited sufficient synergistic action to shorten the clotting time from 40 to 15 sec. The same effect is apparent in curves A and C, using more rapid initial preparations. This effect may be interpreted as due to a reshuffling of components in a thromboplastin complex, or a rebalancing of functionally different thromboplastins. The effect of various clinical conditions on the prothrombin time determined with these thromboplastins is being investigated for possible application to routine prothrombin time determinations.

The practical economic value of this effect is the possibility of increasing the yield of material from one animal several fold, and also of making available a more active preparation of thromboplastin.

These considerations apparently apply also to mixtures of brain thromboplastin. Preliminary experience in this laboratory indicates that mixtures of "deteriorated slow" brain thromboplastin with fast thromboplastin in about equal proportions give prothrombin times equal to those obtained with 100% fast brain thromboplastin. If this is borne out, much thromboplastin, now discarded, may be salvaged.

Chromatographic Analysis of a Mixture of Proteins from Egg White

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Successful separations and analyses of a wide variety of compounds have been achieved with the use of ion-exchange resins (*4*). As a result of the utilization of refractometric optical methods of observation (*2*), the chromatographic separation of protein mixtures has been predicted (*10, 15*). In fact, Tiselius has reported distinct fractionation of protein mixtures on paper by the somewhat different technique of salting-out adsorption (*14*).

This preliminary paper will outline briefly the experimental conditions used for a chromatographic analysis of a protein mixture, and its correlation with the results of an electrophoretic analysis of the same mixture, using the standard Tiselius apparatus (*12*).

The protein system studied, egg white albumin fraction (*5*), was prepared from hen's egg white by the addition of an equal volume of saturated ammonium sulfate and the subsequent removal of the precipitate formed. Just prior to use, the supernatant, which had been stored in the refrigerator, was dialyzed extensively against running tap water and two changes of 20 volumes of cold distilled water. The pH of the final preparation was 6.8.

In preparation for use, the resin, Dowex 50,[1] a cation exchanger in the 200–500 mesh size, was given successive overnight treatments with 4% ammonium hydroxide, 4% sulfuric acid, and 4% ammonium hydroxide, with extensive washing with distilled water between treatments, and a final washing with freshly boiled distilled water. The resin column was formed in one arm of a U-tube which contained a fused-in fine sintered glass disk to support the adsorbent, and was connected at the top to one arm of a standard tall center section Tiselius electrophoresis cell (*7*) through a 1-mm-diam hole in a specially made bottom section. The adsorbent column was 19.5 cm long and 6.9 mm in diam and was covered by a glass wool plug just below the sliding plate interface, connecting bottom and center sections of the modified electrophoresis cell. Just prior to the analysis, the U-tube was washed and filled with protein mixture below the sintered glass disk. During operation, protein was introduced to the bottom of the column through the U-tube from a liquid reservoir above the other vertical arm. The whole assembly, except

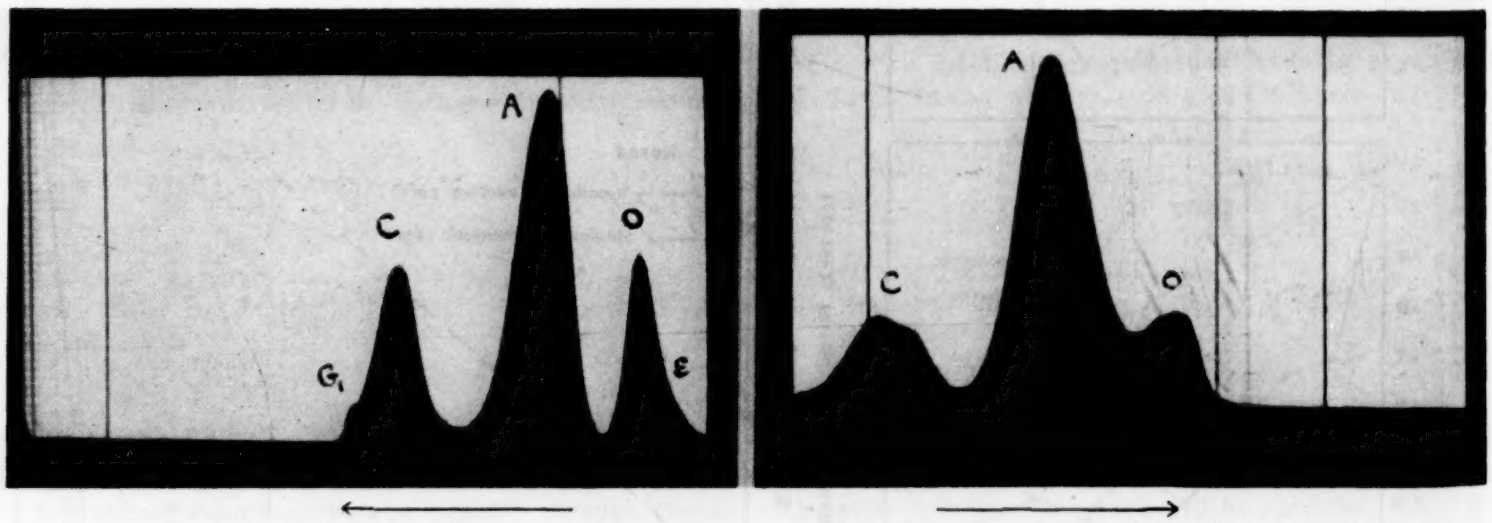

FIG. 1. *Left*, electrophoretic pattern of descending boundaries of egg white albumin fraction in 0.1 M sodium acetate —0.05 M acetic acid buffer at pH 3.90, after 8,520 sec electrolysis at 6.17 v/cm. *Right*, schlieren scanning pattern of effluent from chromatographic column during frontal analysis, flow rate 0.39 ml/hr.

G_1: globulin, C: conalbumin, A: ovalbumin, O: ovomucoid, ε: false buffer boundary. Arrows below patterns represent direction of electrophoretic migration or hydrodynamic flow.

[1] Dow Chemical Company, Midland, Mich.

for the protein reservoir, was immersed in an electrophoresis bath thermostatically controlled at 4° C. Refractometric optical measurements were made on the effluent solution in the Tiselius cell above the column, using the schlieren scanning technique of Longsworth (*6*). This arrangement permits the application of the frontal analysis method of Tiselius and Claesson (*1, 2, 13*).

The electrophoretic analysis of the original albumin fraction is shown in Fig. 1, *Left*. The separation into three major components parallels the results of Longsworth, Cannan, and MacInnes (*9*).

In Fig. 1, *Right* is shown the schlieren diagram of the material emerging from the column during frontal analysis. The separation into three major components is again apparent. By means of a capillary, a sample was drawn from between the second and third peaks. Resolution of this sample in the microelectrophoresis cell (*12*) established the identity of proteins giving rise to the boundaries observed in frontal analysis.

TABLE 1

ANALYSES OF EGG WHITE ALBUMIN FRACTION

Method and sample	Ovomucoid %	Albumin %	Conalbumin %	Total protein concn.*
Frontal analysis (original)	13.2	68.5	18.3	1.95%
Electrophoresis (original)	19.9	56.2	22.8	2.05%†
Electrophoresis (column effluent)	19.7	57.5	21.4	.....

* After water dialysis, assuming specific refractive index increment of 0.00185.

† From freely diffusing boundary.

To investigate the possibility of protein denaturation by the resin, electrophoretic and ultracentrifugal analyses were also performed on material collected from the column after no further chromatographic resolution was indicated by the optical system. The schlieren diagrams obtained from electrophoretic analysis of the effluent reproduced in every detail the patterns obtained from the original egg white albumin fraction, indicating that adsorption onto and desorption from the ion-exchange resin had not altered the charge distribution of the several protein components. However, the ultracentrifuge experiments appeared to indicate that there had been small changes in the size distribution, and this remains to be investigated in more detail.

Comparison of the chromatographic analysis with the electrophoretic analyses of the original albumin fraction and of the effluent from the column is made in terms of percentage composition in Table 1. In addition, the total protein concentration of the sample shown in Table 1 was determined separately from the schlieren scanning diagram of a freely diffusing boundary which had been formed against water and compensated into view in the electrophoresis cell.

Although there is qualitative agreement between the electrophoretic and chromatographic results, it should be noted that both electrophoretic analysis and chromatographic frontal analysis are subject to quantitative errors (*1, 3, 8, 11*), which have not been evaluated for the protein system used here. An investigation of the possible magnitude of the errors involved in the frontal analysis of protein mixtures with this ion-exchange resin is being undertaken by the analysis of synthetic protein mixtures. The feasibility of extending the present technique to larger scale separation of proteins is also under study.

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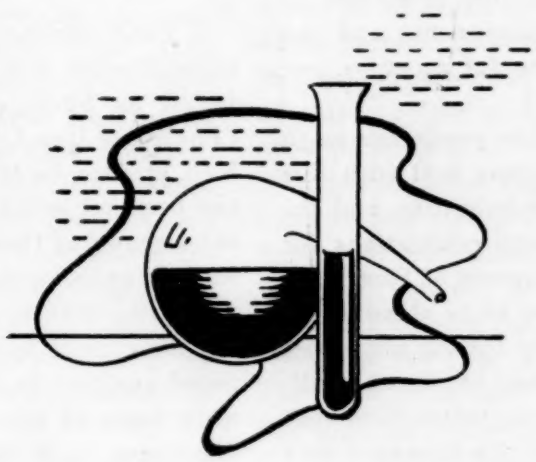

Comments and Communications

The Synthesis of Vitamin B_{12} in the Digestive System of the Sheep

Several months ago it occurred to us that a relationship might exist between cobalt deficiency disease in sheep and the fact that vitamin B_{12} contains cobalt (Smith, E. L. *Nature*, Lond., 1948, 162, 144). Accordingly we fed one sheep 0.4 milligram of cobalt containing radioactive Co^{56}, and a second sheep 1 mg of cobalt containing traced cobalt. On subsequent examination of the feces it was found that more than half of the traced cobalt had been incorporated into an organically bound form. On treatment of the feces with 0.5 *N* HCl almost all the active cobalt could be extracted. On extraction with butanol most of the activity went into the organic solvent in a manner similar to the behavior of B_{12} obtained from liver extracts (*ibid.*, 161, 638). Tests with inorganic cobalt show that a negligible amount passes into butanol from water solution under these conditions. Biological assay, using both *Lactobacillus lactis* Dorner (Shorb, M. S. *Science*, 1948, 107, 397), and *Lactobacillus leichmannii*, indicated the presence of large amounts of vitamin B_{12}. Thus, sheep feces appear to be an important source of B_{12}.

We have seen the paper of L. S. Gall *et al.* (*Science*, 1949, 109, 468), showing that the growth of certain bacteria in rumen of the sheep is stimulated by the administration of cobalt. It is tempting to assume that these rumen bacteria synthesize the B_{12}.

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Scientific Research vs. the Theory of Probabilities

There is an increasing tendency to force use of the theory of probabilities upon those engaged in scientific research. To me, scientific research is the attempt to discover and establish principles for accurate prediction of what will happen. Can we use measurements and the absolute truths of the mathematicians for accurate prediction in human or biological affairs?

To whom should one go for accurate prediction as to how long one will live? Mathematicians deal with this subject by means of the theory of probabilities, and the actuaries they train make the necessary calculations for life insurance companies. For the purposes of these companies one goes to a medical examiner to be classified as to length of life. However satisfactory for the companies these calculations and classifications may be, for the individual case the prediction may seem no better than that made by an astrologer. I was refused life insurance over twenty years ago, and the other day a neighbor was accepted for life insurance in the morning and died going upstairs in the afternoon!

The prestige of mathematics is so great that many persons forget that even in mathematical hands, *probability, chance,* and *random* mean ignorance. They come to think that, in the alembic of mathematics, chance in some way becomes certainty. They take great care to select random samples without realizing that, insofar as a sample has been random, they don't know how it was selected.

The biologist's greatest gift from mathematics might well be, not a theory that may delude him into belief that he is wise when he is ignorant, but rather the ideal of clear definition and precise use of his terms and symbols, not excepting *science* and *research*. When we are faced with discrepant results in our handling of facts, four courses are open to us. First, we may gloss over our failure in prediction by saying that the exception proves the rule. Second, we may abandon our principle of prediction and fold our hands. Third, we may hold onto that principle and by piling up results and treating them mathematically try to show accurately, for intellectual satisfaction or for practical action, just how much or how little the principle determines what happens. Scientists who content themselves with testing theories or supposed principles can well use the theory of probabilities and may call this scientific research. To go no further is to abandon the search for new principles that may permit accurate prediction in the individual case.

Finally, we may be stimulated by the discrepancy between our results and our expectations to discover unknown principles; this will be true scientific research. It is to be contrasted with, not assisted by, use of the theory of probabilities. The latter is a most valuable tool for practical action on the basis of current knowledge and current ignorance.

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Name of the Soybean

J. Paclt (*Science*, 1949, 109, 339) has proposed that the name *Glycine Max* (L.), as used for the soybean, be rejected as having been based on a *nomen confusum* (*Phaseolus Max* L.) and that the name *Glycine Soja* (L.) Sieb. et Zucc. be taken up in its place. Perhaps no plant has been subjected to more nomenclatorial buffeting and name-changing than has the soybean—a situation that always is unfortunate, and the more so for a plant of economic importance. In a more recent extensive accounting for the correct name of this plant I have presented detailed analyses to support the contention that the legitimate name of the soybean is *Glycine* Max (L.) Merrill (Lawrence, G. H. M. *Gentes herbarum*, 1949, 8 fasc. 1.)

The name of the soybean dates from 1753 when, in his

Species plantarum, Linnæus designated it as *Phaseolus Max*. The description he gave is of itself inadequate. Paelt alluded to the presence in this description of "some specific characters derived from another element, namely *Phaseolus Mungo* L." In the absence of specific details in support of his claim, it is indeed hazardous to accept his contentions and, contrary to his statement, I know of no contemporary botanists who treat the mung bean as conspecific with the soybean. Offsetting this deficiency in his description of 1753, the earlier references cited by Linnæus and the available type specimen of the plant make clear the identity of the soybean. Careful study of them fails to indicate the basonym of *Phaseolus Max* L. to be a *nomen confusum*. The specimen of *Phaseolus Max*, on which Linnæus based his name, was provided him by George Clifford, and is currently reported to be in the Linnæan herbarium. The more ample description by Linnæus in *Hortus Cliffortianus* (1738) is presumed to have been based on the same Clifford specimen, and this earlier account may serve to supplement the inadequate diagnosis in *Species plantarum*.

It is the opinion of Paelt (*loc. cit.*) and, for wholly different reasons, of Hill (*Bot. Mus. Leaflets Harvard Univ.*, 1939, 7, 107) that the name of the soybean is *Glycine Soja* (L.) Sieb. et Zucc. The name as used contemporarily, and not originally by Siebold and Zuccarini, was based on *Dolichos Soja* L. As was true of *Phaseolus Max*, Linnæus provided only a fragmentary description of *Dolichos Soja* in his *Species plantarum*, but cited his earlier and identical description as given in the *Flora Zeylanica* (1747). This earlier description was based on a specimen collected from cultivation in Ceylon by Paul Herman prior to 1677. After Linnæus' time the wild indigenous prototype or counterpart of the soybean became known to science. Moench (1794) considered it distinct from the cultigen and named it *Soja hispida*. In 1845 Siebold and Zuccarini treated the same plant under the new name of *Glycine Soja*. This is a case involving two different types of specimens collected from two divergent geographic regions: *Dolichos Soja* L. from cultivation and *Glycine Soja* Sieb. et Zucc., an indigen. Other early botanists considered the two plants to be different entities; later botanists have treated them as conspecific. However, by Article 18 of the Rules of Botanical Nomenclature, we are not allowed to take up a name based on a different type from that accepted by the author of the name. Siebold and Zuccarini clearly excluded Linnæus' *Dolichos Soja* from their concept of *Glycine Soja*. It is most unfortunate that they chose the name *Soja* for their plant. Because of these circumstances it is incorrect to cite Linnæus as a parenthetical author of their binomial.

I have attempted to refute Paelt's contention, unsupported by requisite data, that *Glycine Max* (L.) is based on a *nomen confusum* and to show that in no case is the name *Glycine Soja* Sieb. et Zucc. available as a legitimate name for the soybean. It seems clear to me, until such time as the case may be reviewed and an opinion given by more competent authority, that we should continue to designate the soybean as *Glycine Max* (L.) Merrill.

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A Six-Segment Head Regenerate in a Supposedly Refractory Earthworm Species, *Lumbricus castaneus* Savigny 1826

It has been shown (Carpenter, E. *Science*, 1948, 108, 625), that, contrary to general belief, a head of six segments may be regenerated in the manure worm, *Eisenia foetida* (Savigny) 1826. This species, in proper laboratory conditions, regenerates readily and rapidly. *Lumbricus castaneus*, however, has been thought to have little or no regenerative capacity, presumably because of Hescheler's failure to secure regeneration (*Z. Nat.*, Jena, 1896, 30, 177).

Material was secured from a pile of old leaves behind a Harvard building. Experimental conditions were the same as for *E. foetida* (Gates, G. E. *Biol. Bull.*, 1949, 96, 129), except that in this case all regeneration was terminated at 30 days. The species has been found only twice in the U. S., and inability to secure further material ended the experiments.

All posterior substrates with transections at levels from 4/5 to 7/8 inclusive survived and regenerated (no operations behind 7/8). Regenerates at 4/5 or 5/6 had little or no metameric differentiation. Regenerates at the next two levels were normally cephalic, of three (1 specimen) and four segments (1) at 6/7, and at 7/8 of six (1) and 5½ (1) segments. In the latter case the half-segment was wedge-shaped and on the left side. The prostomium of each regenerate, apparently completely differentiated, was epilobic, rather than tanylobic as supposedly characteristic of the genus *Lumbricus*.

Regeneration of a normal head of six segments at 7/8 enables prediction of a species capacity to regenerate equimeric heads at 6/7 and all levels anteriorly.

A six-segment-head regenerate from such a limited number of operations, on a supposedly refractory species, seems to warrant another prediction, namely, that further investigation will show that the capacity for head regeneration, throughout the family Lumbricidae, has been underestimated.

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Determination of Condition of Oysters

It is difficult to devise a method of evaluating the condition of an organism by making analyses of only a few of the factors concerned. A recent publication by Robert M. Ingle (*Science*, 1949, 109, 593) illustrates the nature of this problem in the extensive researches now being made on oysters.

Ingle mentioned that "later workers have adopted the measurement of glycogen content as a supplementary method of evaluation," meaning supplementary to the "index" method, as explained herein, which was developed by the writer and published in brief form in 1938 (in Higgins, E. *Rep. Commis. of Fish*, 1937). The glycogen method is the traditional one and has been employed by various investigators—P. H. Mitchell (*Bull. U. S. Bur. Fisheries*, 1917, 35, 151), P. S. Galtsoff *et al.* (*Bull. U. S. Bur. Fisheries*, 1935, No. 18), and others.

It was because of the apparent unreliability of the glycogen method that the more recent index procedure was put into use.

Ingle objected to the index method because his graph, in which he plotted index values against percent of glycogen, did not indicate a uniform relationship. The figure clearly proves his point, and in doing so should suggest the obvious conclusion that there is no reason for expecting the two methods to give comparable results. It would be very surprising should they do so, in which case the need for the index method would not have arisen.

In applying this method, calculations are also made of the ratio of meats undrained and drained to the volume of the shell cavity. However, it has been found consistently that, because of osmotic effects from changes in salinity, the condition of an oyster, or of a group of oysters, as usually employed, may well be expressed by the equation referred to by Ingle, namely,

$$\text{Index} = \frac{100 \times \text{Dry weight (g)}}{\text{Volume shell cavity (cc)}}$$

The index method was specifically designed to give a picture, not of the glycogen cycle throughout the year, but of the actual quality of meat when reduced to a dry basis, in order to eliminate errors due to osmotic effects, maturation of gonads, and spawning.

In further support of this point it is of interest to mention the following figures on analyses of oyster meats (Chatfield, C., and Adams, G. *U. S. Dept. Agr. Circular No. 549*, 1940): "protein, 9.8%; fat, 2.0%; carbohydrate (glycogen), 5.9%." These figures definitely refer to drained oysters during the winter season as used commercially, but they indicate that glycogen constitutes only about one-third of the tissue substance. The method of glycogen analysis ignores proteins completely, though they are nearly double the glycogen; while the index method measures the entire amount of meat.

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Effect of Dioxane and Sodium Hydroxide upon Lens Capsule and Cortex (*Squalus* sp.)

The use of these chemicals in conjunction with the study of the lens may complement certain laboratory activities in physiology and comparative anatomy. To demonstrate specific peculiarities of the lens the following procedures can be followed during the regularly scheduled laboratory periods without interrupting the prescribed requirements.

A certified grade of dioxane should be used for optimum clearing of the lens structures. Appreciably fine results may be obtained from salvaged dioxane, if it is dehydrated with CaO and filtered before using. Dioxane and water are miscible in all proportions, but a more uniform infiltration occurs if the lenses are suspended in the medium rather than allowed to rest on the base of the vial. This caution should be observed if reclaimed dioxane is used.

The type of lens utilized in these procedures may be obtained from the commercially preserved dogfish. The lens must be carefully removed from the eye to avoid injuring the lens capsule. It is then immersed in the dioxane for 10 min, removed, and air-dried.

A lens so treated will appear as a clear, pale amber, uniformly homogenous structure. In this condition it is impossible to distinguish the capsular, cortical, or nuclear components because of their uniform transparency. Very faint striations and suture lines may, however, be observed on the surface of the capsule with the aid of a hand lens. This preparation, because of its clarity, serves most efficiently for the demonstration of the refracting and focusing powers peculiar to lenses.

If the capsule is mechanically scratched or broken, and the specimen again immersed in dioxane for 25 min, white opacities, comparable to cataracts, appear upon the cortical surface. Excessive exposure to dioxane will render the entire specimen white.

Further observations pertaining to the cortex may be facilitated by the use of 20% NaOH solution. The lens should be placed in the alkali for 15 min, then washed and dried. This treatment will clearly reveal the capsular suture lines.

If the alkaline-treated lens is observed before drying, the lamellated character of the slightly swollen cortex is readily seen; and it may easily be peeled. The removal of the cortical strata will expose the nucleus, which is a transparent, amber, homogenous unit, free of striations and lamellations.

By holding the moist lens against the light before removing the cortex, the shiny nucleus may be seen almost centrally located within the gelatinous cortical envelope.

Prolonged exposure of the lens to the alkali, 24 hr, will cause a gross swelling of the cortex and of the nucleus, and a change from the clear state to a uniformly hazy condition.

This procedure provides a means of more completely utilizing the lens when the structure of the eye is studied in comparative anatomy; and a means, also, of demonstrating the refractive powers, cataracts, and other lens characteristics in physiological applications.

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Book Reviews

Economic geography of the USSR. S. S. Balzak, V. F. Vasyutin, and Ya. G. Feigin. (Eds.) New York: Macmillan, 1949. Pp. xlv + 619. (Illustrated.) $10.00.

This is a volume which opens with a quotation from Stalin, and closes with reference to polar fliers who "are ready, if it becomes necessary, to transfer from civilian to military planes and destroy the enemy, wherever he may be." Between these two items, unusual in a volume of economic geography, are four comprehensive chapters on natural resources, industry, agriculture, and transport as seen by the geographer, plus three ideological treatises on the Soviet interpretation of production and population under tsar and socialism.

Economic geography of the USSR is nonetheless valuable if it combines excellent geography with partisan ideas, for the Soviet lands cannot be understood without both. With major reservations, this promises to be the definitive volume on Soviet geography. It was originally published in 1940, largely with 1935 data, and is here translated under the auspices of the American Council of Learned Societies with the editorship of Chauncy D. Harris. This is the only comprehensive geography of the Soviet Union ever to be published in Russian and its translation is a major event. Fifty-three tables, 83 maps, and six appendices provide a wide array of information nowhere else available; in fact, the American translators have materially enriched the original volume.

It is now clear that the USSR is second only to the USA in the wealth of its resources:

> Thus, the USSR has diverse natural conditions and natural riches such as are possessed by no other country in the world. "From the standpoint of natural wealth, we are completely secure. We have even more than we need." But in order to put these natural riches completely into the service of the working people, in order to create an abundance of all kinds of products, "there must be a government with the desire and the power to direct the utilization of this huge national wealth for the benefit of the people. Do we have such a government? We do."

(Quotation from Stalin.) What is also clear, although not stated by the authors, is that limitations of cold, drought, and continentality place permanent restrictions on economic developments. One might suggest that in academic terms, the best "grade" the Soviet lands may hope to receive under any conditions of government is no better than an A–. This is, to be sure, a creditable rating, although not yet achieved.

Two sentences, chosen at random, reflect Soviet thinking:

> The capitalist town subjugated the village and artificially retarded its cultural development. For this reason, in the eyes of the peasants the town was always the focus of their exploitation. . . . The Marxist-Leninist understanding of the role of the natural-geographic environment has nothing in common with crude geographic theories . . . as explained by bourgeois geographers and economists. . . .

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Veterinary helminthology. Banner Bill Morgan and Philip A. Hawkins. Minneapolis 15, Minn.: Burgess Publ., 1949. Pp. ix + 400. (Illustrated.) $7.00.

Everyone who deals with animals knows that there is a whole world of parasitic life associated with them. Probably everyone but a specialist with a bulging reprint library, however, would be astonished to know what an extraordinarily abundant and diverse part of this fauna the helminths represent. Morgan and Hawkins, in their text and reference book, have undertaken the formidable task of digesting this literature with reference to "animals of veterinary importance in North America" for the first time. The result is an impressive 400-page volume of encapsulated information, concerning both hosts and worms, species by species.

Following a general introduction (36 pp.) there are chapters on the helminths of the horse (32 pp.), of cattle (38 pp.), of sheep and goats (55 pp.), of swine (36 pp.), of the dog and cat (57 pp.), of poultry (45 pp.), of fur bearers (44 pp.), and on diagnosis (20 pp.). There is an appendix, principally of host lists (13 pp.), and a comprehensive index (14 pp.).

The authors deal with over 130 genera and about three times that many species. Drawing on an extensive teaching experience, they present for each species selective information—so far as possible—on synonyms, common name, disease, morphology, life history, symptoms, pathology, diagnosis, treatment, and control. Supplementing the text are keys for identification of various groups and life history stages, and 63 plates of line drawings and maps. From the degree of attention accorded them, the parasites of greatest interest in this field in America are *Fasciola hepatica, Moniezia expansa, Oesophagostomum columbianum, Haemonchus contortus, Trichostrongylus colubriformis, Ascaris lumbricoides, Trichinella spiralis, Ancylostoma caninum,* and *Dirofilaria immitis.* Besides emphasis on the veterinary problem, as such, relationships between parasitized domestic and wild animals and helminthic infections of man are considered.

The volume is a useful encyclopedic work, although it shows, as a first edition, certain rough spots in phrasing and proofreading. One is tempted even in a general review to raise a few questions, however. An idea tacit throughout the presentation seems debatable in our present understanding of worm-host relations—namely, that "an animal infected with worms is *suffering* from helminthiasis" (italics by reviewer). The verb implies as a rule more than is frequently true physiologically. Immunizing infections with worms are briefly discussed, and whereas up to now artificial immunization has not been as fruitful a procedure with helminths as with certain other parasitic agents, it is scarcely correct to say that "the introduction of suspensions of helminths or dead helminths into the host has not resulted in the production of any immunity" (p. 7). One misses a chapter on the

ever present problem of cross infection of hosts capable of parasitization by the same helminthic species. Compartmentalization host by host is a marked convenience for discussion, but doesn't adequately reflect the way of nature. For instance, horses, cattle, sheep, and goats all harbor the minute *Trichostrongylus axei*, which may on occasion have its degree of pathogenicity obscured by attention centered on the larger helminths. Do these hosts "suffer" equally by infection with this nematode? If unequally, is the placing of horses in infested cattle or sheep pastures a danger, or means of control? Is the reverse order of pasturing advisable?

In regard to cross infections to man, the indication that *Taenia saginata* (p. 80) may cause human cysticercosis as a blind alley infection with a facility equal to that of *T. solium* (p. 167) is unwarranted, a point especially unfortunate as to cerebral cysticercosis, in which the pork tapeworm alone has been implicated. Moreover, the statement that for *Hymenolepis nana* "rodent and human forms are interchangeable" (p. 337) is counter to currently available evidence. If there were no other domestic animal reservoir of helminthic infection for man than the hog for *Trichinella spiralis* we would still have a challenging public health problem, and there is need for caution in emphasizing forms of no demonstrable threat.

Such errors have a tendency to emerge where authors so obviously strive toward simplification of statement in a complex subject. It is easy to overdo simplification and the unqualified declarative sentence strikes this reviewer as having been rather too freely employed. What is wrong with the occasional use of the interrogation point in textbooks?

The helminths of domestic animals are going to be with us for a long, long time. And, as an experimental area, veterinary helminthology offers unsurpassed opportunities to explore biological and practical angles of worm-host relationships. Not a few of these can contribute to central problems in which the human host has a very great interest indeed. Morgan and Hawkins have helped specifically to open these areas to further exploration.

NORMAN R. STOLL

Rockefeller Institute for Medical Research,
Princeton, New Jersey

Personal adjustment in old age. Ruth Shonle Cavan, Ernest W. Burgess, Robert J. Havighurst, and Herbert Goldhamer. Chicago: Science Research Associates, 1949. Pp. xiii + 204. $2.95.

The purposes of this volume are (1) to define and analyze the nature, pattern, and problems of individual adjustment to aging; (2) to present certain facts about old age obtained from census data and a special survey by the authors; and (3) to describe two questionnaires used for measuring the adjustment of the senescent and senile. Its contents are of particular interest to physicians, psychologists, sociologists, and social workers. The authors have neither pioneered in an entirely new field nor have they produced a definitive work, but theirs is a valuable contribution to the rapidly growing literatu on geriatric sociology.

Problems of adjustment in old age and the techniqu for developing a valid instrument for their measureme are discussed in considerable detail. Two instrumen devised by the authors, an "Inventory of Activities" an an "Index of Attitudes," although admittedly imperfe are the basis for much of the data in this study. Th statistical treatment is thorough but a succinct summar of results is lacking. The check list for the detection o neuroses is of questionable value, since it lists man symptoms also characteristic of the organic disorder frequently found in elderly persons. In the discussio of adjustment cycles in old age it is assumed that durin middle age the individual has been both personally an socially well adjusted. This assumption is, of cours entirely unwarranted and ignores the continuity of emo tional difficulties from one period of life into the nex The "Index of Senility" is open to serious criticism, fo it contains a large number of nonspecific items which might be misconstrued. In summary, *Personal adjustment in old age* is an interesting book, but it should be read critically rather than taken at its face value.

S. T. KIMBL

Washington, D.C.

Mathematical foundations of statistical mechanics. A. I. Khinchin. (Trans. from the Russian by G. Gamow.) New York (19): Dover Publs., 1949. Pp. viii + 179. $2.95.

Introduction to statistical mechanics. G. S. Rushbrooke. New York: Oxford Univ. Press, 1949. Pp. xiii + 334. $5.50.

These two books stress different aspects of statistical mechanics, and they are written for two different groups of readers. Khinchin's book is written primarily for mathematicians, while Rushbrooke's text is intended for students of physical chemistry.

The main interest for a physicist in Khinchin's book will probably lie in two points. The more important of these is Khinchin's discussion of the ergodic problem. The second point is his discussion of the order of magnitude of the terms usually neglected. The importance of this can be seen from Schubert's recent criticism of Gentile's intermediate statistics (*Z. Naturforschung*, 1946, **1**, 113).

It is, however, to be doubted whether Khinchin's book will find a wide audience, apart from mathematicians wanting to get acquainted with statistical mechanics, or physicists wishing to brush up their knowledge of the ergodic problem. The book is written without a physical background and deals only with classical statistic mechanics, while it might have been hoped that this treatise could have supplemented the masterly analysis of the Ehrenfests in the *Enzyklopaedie der Mathematischen Wissenschaften.* However, the typical quantum mechanical problems are not discussed at all, and the footnote on page 51 shows that Khinchin's understanding of these problems is limited. A nearly total lack of references

makes the book very impractical to use. For instance, it is nowhere stated where one can find the important Birkhoff theorem—the center piece of the whole book, in my opinion. For the interested reader, the reference is: *Proc. nat. Acad. Sci.*, Wash., 1931, **17**, 656.

Dr. Gamow's own Russian background at times peeps around the corner, e.g., when the name Birkhoff is also transcribed (from the Russian transcription) as Virkoff on page 27. To call the ideal gas law Clapeyron's law (p. 121) should not have been permitted by Dr. Gamow, who must know that Clapeyron was only 3 years old when Gay-Lussac first announced his law in 1802.

Rushbrooke's book can be highly recommended as a textbook for intermediate courses in statistical mechanics. It is comparable to Mayer and Mayer's book, but is not as extensive as this widely used text. The mathematics is kept simple and a great number of exercises at the end of each chapter greatly increase its usefulness.

For the sake of simplicity, the author has given up rigor. Characteristic of the tenor of the book is the author's statement in a footnote on p. 58: ". . . this naive statement is not seriously misleading, and conveys the correct idea."

It is a great pleasure to see a text on statistical mechanics which strongly advocates the use of Gibbs' grand ensembles. In this connection it is strange that on page 268 de Boer's work is not mentioned explicitly. The absence of Fowler's monograph in the list of standard treatises is rather surprising, since it is practically the only text referred to in British papers in the field. It is strange to me that the old quantum mechanical statistical mechanics is used, instead of the Fermi-Dirac and Bose-Einstein statistics, as the basis of this treatise and as a basis for the subsequent transition to classical statistics—but this is certainly a minor point, which does not affect the great value of Rushbrooke's text for those students who wish to get acquainted with the basic ideas of statistical mechanics.

D. TER HAAR

Purdue University

Association Affairs

The New York Meeting, December 26–31, 1949

The Science Theater

The AAAS Science Theater, now a permanent feature of the Association's annual meetings, will show recent scientific films almost continuously throughout the meeting period. Hours are: December 26, 2:00 p.m. to 6:00 p.m.; December 27, 9:00 a.m. to 1:00 p.m. and 2:00 p.m. to 10:00 p.m.; December 28, 29, and 30, 9:00 a.m. to 1:00 p.m. and 2:00 p.m. to 6:00 p.m.; December 31, 9:00 a.m. to 1:00 p.m. Seating is at any time. The schedule has been so arranged that programs given in the first half of the week are repeated in the second half, and shifted from morning to afternoon, to give registrants another chance to see films that interest them.

The Science Theater is on the 18th floor of the Hotel Statler, just back of the express elevators. The capacity of this room is limited, so that admission, although free, is restricted to registrants. Showing a registration receipt will do, but wearing a badge will save time.

The Association is greatly indebted to all those who made these pictures and lent them for showing. Special appreciation is due the First District Dental Society of New York for making available its lecture room and screen.

The schedule follows:

Program 1

Monday afternoon, December 26

2:00 p.m.–6:00 p.m.

1. *Nagana*—South African Scientific Liaison office. Sound, color, 25 minutes.
2. *Anterior Dislocations of the Shoulder*—Davis & Geck, Inc. Silent, color, 41 minutes.
3. *Meiosis*—Arthur T. Brice. Sound, black and white, 19 minutes.
4. *Safety in the Chemistry Laboratory*—Indiana University. Sound, black and white, 15 minutes.
5. *Solar Prominences*—University of Michigan. Sound, black and white, 11 minutes.
6. *Conquest of the Hudson*—Port of New York Authority. Sound, black and white, 20 minutes.
7. *Then It Happened*—U. S. Forest Service. Sound, color, 10 minutes.
8. *Avian Cecal Coccidiosis*—Ohio State University. Silent, color, 30 minutes.
9. *Bound for the Caribbean*—Royal Dutch Air Lines. Sound, color, 45 minutes.

Program 2

Tuesday morning, December 27

9:00 a.m.–1:00 p.m.

1. *Amputation Prostheses and Their Uses. I*—Medical Section Headquarters, 1st Army, Governor's Island, New York City. Sound, black and white, 34 minutes.
2. *Story of DDT*—British Information Services. Sound, black and white, 25 minutes.
3. *Radar Detection of Storms Occurring in the New England Area*—Massachusetts Institute of Technology. Silent, black and white, 20 minutes.
4. *Stepping Along with Television*—American Telephone and Telegraph Company. Sound, black and white, 11 minutes.

5. *Brownian Movement*—Army Chemical Center. Silent, black and white, 15 minutes.
6. *Story of Palomar*—California Institute of Technology. Sound, color, 40 minutes.
7. *Skin Antiseptics*—Chilean Iodine Educational Bureau. Sound, black and white, 30 minutes.
8. *Cell Division*—Arthur T. Brice. Sound, black and white, 16 minutes.
9. *Surgical Approach for Hypertension*—Garfield Memorial Hospital. Sound, color, 20 minutes.
10. *Colour*—British Information Services. Sound, color, 14 minutes.

Program 3

Tuesday afternoon, December 27

2:00 p.m.–5:00 p.m.

1. *Image Dissector Motion Pictures at Ten Million Frames per Second*—University of Rochester. Silent, black and white, 15 minutes.
2. *Amputation Prostheses and Their Uses. II*—Medical Section Headquarters, 1st Army, Governor's Island, New York City. Sound, black and white, 34 minutes.
3. *Story of Lubricating Oil*—U. S. Bureau of Mines. Sound, color, 21 minutes.
4. *Charting the Seas*—British Information Services. Sound, black and white, 24 minutes.
5. *Crystal Clear*—American Telephone and Telegraph Company. Sound, color, 20 minutes.
6. *Studies of Specificity of Nonhemolytic Streptococci in Relation to Idiopathic Epilepsy, Schizophrenia, Encephalitis and Poliomyclitis*—E. C. Rosenow. Silent, black and white, 20 minutes.
7. *Principles of Electricity*—General Electric Company. Sound, black and white, 40 minutes.
8. *On Time and Light*—Henry M. Lester. Silent, color, 20 minutes.
9. *The Hurricane Circuit*—U. S. Department of State.

Program 4

Tuesday evening, December 27

6:00 p.m.–10:00 p.m.

1. *Artificial Insemination of Rabbits and Transplantation of Rabbit Eggs*—Worcester Foundation. Silent, color, 20 minutes.
2. *Gift of Green*—Sugar Research Foundation. Sound, color, 20 minutes.
3. *Life History of the Rocky Mountain Wood Tick*—U. S. Public Health Service. Silent, color, 45 minutes.
4. *Jet Propulsion*—General Electric Company. Sound, color, 15 minutes.
5. *Wyoming and Its Resources*—U. S. Bureau of Mines. Sound, color, 30 minutes.
6. *Conquering the Jungle*—Goodyear Tire & Rubber Company. Sound, black and white, 10 minutes.
7. *Amputations for Occlusive Arterial Disease*—Davis & Geck, Inc. Silent, color, 30 minutes.
8. *Project 5-4040, High-speed Studies of Safety Glass*—Monsanto Chemical Company. Sound, color, 18 minutes.
9. *Exploring with X-Rays*—General Electric Company. Sound, black and white, 40 minutes.

Program 5

Wednesday morning, December 28

9:00 a.m.–1:00 p.m.

1. *Highway to Alaska*—Allis-Chalmers Manufacturing Company. Sound, color, 23 minutes.
2. *Millions for Safety*—Port of New York Authority. Sound, black and white, 10 minutes.
3. *This is Nylon*—E. I. du Pont de Nemours & Company. Sound, color, 29 minutes.
4. *Timber and Totem Poles*—U. S. Forest Service. Sound, color, 10 minutes.
5. *Voice Sentinel*—American Telephone and Telegraph Company. Sound, black and white, 16 minutes.
6. *Nevada and Its Resources*—U. S. Bureau of Mines. Sound, color, 31 minutes.
7. *The Story of Tin Plate*—U. S. Bureau of Mines. Sound, color, 21 minutes.
8. *They Also Serve*—American Medical Association. Sound, black and white, 15 minutes.
9. *Pacific Halibut Fishing*—U. S. Fish & Wildlife Service. Sound, color, 12 minutes.
10. *Application of Cinefluorography*—R. F. Rushmer. Silent, black and white, 10 minutes.
11. *One Second in the Life of a Hummingbird*—New York Zoological Society. Silent, color, 15 minutes.
12. *Baby from Borneo*—New York Zoological Society.
13. *This is Their Story*—Film Program Services (Unesco). Sound, black and white, 20 minutes.

Program 6

Wednesday afternoon, December 28

2:00 p.m.–6:00 p.m.

Same as Program 2.

Program 7

Thursday morning, December 29

9:00 a.m.–1:00 p.m.

Same as Program 1.

Program 8

Thursday afternoon, December 29

2:00 p.m.–6:00 p.m.

Same as Program 4.

Program 9

Friday morning, December 30

9:00 a.m.–1:00 p.m.

Same as Program 3.

Program 10
Friday afternoon, December 30
2:00 p.m.–6:00 p.m.
Same as Program 5.

Program 11
Saturday morning, December 31
9:00 a.m.–1:00 p.m.
Selections from Programs 1–5.

NEWS and Notes

Reports on Antiseptics Conference, New England Geologists' Meeting, and Optical Society Meeting

A Conference on Mechanism and Evaluation of Antiseptics was held October 28–29 under the sponsorship of the Section of Biology of The New York Academy of Sciences. A registered attendance of 518 was reported by Herbert L. Davis, of the Ethicon Suture Laboratories, conference chairman.

The purposes of the conference were: (a) to summarize and evaluate existing information on the mode and extent of antimicrobial agents *in vitro* and *in vivo*, and (b) to reveal those avenues of investigation likely to produce more active compounds and more effective application of them. Perhaps the most significant outcome of the conference was the general acceptance of the view that the action of antimicrobial agents is governed significantly, if not primarily, by the principles of colloid chemistry, whether these agents be rapidly lethel disinfectants, skin antiseptics, or chemotherapeutic drugs. Living organisms are colloid structures, and antimicrobial substances of both biological and synthetic origin must first be adsorbed on or in the organism. Although some of the mechanisms of antimicrobial action, such as protein coagulation and poisoning of essential enzyme systems, are well recognized, others are only suggested by present evidence. Of particular interest is the observation made in several separate studies that adsorbed substances alter the permeability of the cell wall, causing release of bacterial protein, other nitrogenous materials, and electrolytes. Thus, the cell is no longer in equilibrium with its environment. It became increasingly clear during the sessions that empirical testing of compounds should yield to a systemic and rational study of the mechanisms by which existing antimicrobial substances act.

The 25 papers presented at the conference dealt with a wide variety of compounds, including antibiotics, cationic, anionic, and nonionic agents of high surface activity, halogens, heavy metals, and ethyl alcohol. Several authors emphasized the frequent lack of correlation between results with antiseptics *in vitro* and *in vivo*. Because results *in vitro* are frequently false due to inadequate test conditions, considerable discussion concerned the need and continued search for better antiseptic neutralizers with which to distinguish between bacteriostatic and bactericidal effects. It was generally agreed that once activity *in vitro* is established the crucial tests are those which simulate actual clinical use. Here toxicity to tissue is of primary importance, but there is still disagreement as to the proper type of tissue to use. One new procedure which directly measures the prevention of sepsis *in vivo* was presented at the conference and well received.

Microbial populations are heterogeneous in that the constituent cells possess varying degrees of resistance. This is not a new concept, but one too frequently ignored in the field of disinfection. One paper at the conference explored the nature of this variation and the factors influencing it; another proposed that the commonly applied all-or-none end point be replaced by a less severe criterion of antiseptic usability, the count of surviving organisms.

This report would be incomplete without mention of the fact that the value of ethyl alcohol as an antiseptic and disinfectant was reaffirmed and that the addition of antiseptics to 70 percent ethanol failed in some instances to increase activity. Another point of interest was the reported antiviral activity of several types of antiseptics.

There emerged from the conference a clear recognition that a whole panel of tests are necessary to establish the value of an antiseptic, and that the greatest emphasis should be placed upon those procedures *in vivo* which measure prevention of sepsis. It was equally apparent, however, that many fundamental problems remain almost untouched. It is not known, for example, whether the same or different mechanisms are involved when bacteria are rapidly killed by a strong concentration but merely inhibited by a weaker one. Nor was any new information presented as to how bacterial spores are destroyed.

EARLE H. SPAULDING

New England Field Geologists. More than 165 geologists, representing 20 colleges, attended the 42nd annual field meeting of the New England Intercollegiate Field Geologists, meeting October 14–16, 1949. Robert L. Nichols and Charles Stearns, Department of Geology, Tufts College, were hosts and were assisted by Marland P. Billings, Harvard University, L. W. Currier, U. S. Geological Survey, and Robert Shrock, Massachusetts Institute of Technology.

Prof. Shrock led a group on Friday afternoon to study the critical exposures of the well-known Squantum tillite complex which is, in places, underlain by similar rock in which thin bedding, interpreted as varves, is developed. Other microstructures in tillites and associated stratified rocks were studied.

The lithology and stratigraphy of the area surrounding the Chemsford granite were discussed at selected points by a group led on Saturday by L. W. Currier. Problems of granitization and the relative degrees of "invasive metamorphism" were noted. The stratigraphic sequence of rocks in the area was noted, and it was illustrated on the trip that 1) pebbles of the Merrimack quartzite (oldest in series) occur in the overlying Harvard conglomerate; 2) that the Harvard conglomerate, at the base of the Worcester phyllite, should be considered a local member of the phyllite; 3) that this phyllite grades upward into the Brimfield schist; and, that the schist grades upward into a paragneiss. Correlation of the Merrimack with the Oakdale quartzite farther southwest and with the Kittery quartzite to the northeast was suggested. Characteristic outcrops of the several formations and the border zone of granitized quartzite were studied.

Profs. Nichols and Stearns led a large group on Saturday to study bedrock geology, glacial geology, and shoreline geomorphology in the area of the north shore from Winthrop and Ipswich. Recently built tombolos near Winthrop, drumlins northeast of Boston, and deposition of flying bars were observed. Peat deposits, older than the present beach deposits, were studied near Revere Beach. Blue Clay deposits, with Greenland fossils, resting beneath beach sands and gravel in some areas, indicated a previously higher stand of the sea. On Nahant, the group examined Cambrian metamorphosed sediments, including diabase sills, folded into synclinal structures and invaded by gabbro.

Another large group, led by Prof. Billings, studied the stratigraphy of the Boston Basin in the vicinity of Nantasket on Sunday. Interbedded basic tuffs and basic flows constituting the north limb of a syncline were examined and conglomerate outcrops on the south limb of the anticline were visited. It was possible to observe that the conglomerates fingered out northward into volcanic rocks. In places, the Roxbury conglomerate rests uncomformably on the Dedham granodiorite and encloses large (6-ft diameter) blocks of the Dedham. The leader pointed out evidence to sustain the thesis that the Roxbury was deposited on a surface of perhaps 500-ft relief in the Nantasket area and 2500 ft in the Hingham area. The evolution of Nantasket Beach was discussed by Nichols, who pointed out wave-cut cliffs now one-quarter mile landward from present sea level.

At the regular business session of the group, held in Barnum Museum, Tufts College, the 1950 field trip was assigned to Joseph Trefethen, head of the Maine University Department of Geology at Orono.

LLOYD W. FISHER

OSA at Buffalo. Optical microscopes and microscopy received major attention at the 34th annual meeting of the Optical Society of America, which was held at the Hotel Statler, Buffalo, New York, on October 27, 28, and 29. Five excellent invited papers were followed by a group of contributed papers on aspects of microscopy, and gave the listeners a very well-rounded picture of the distinguished history, current practice, and new frontiers of this important branch of applied optics.

Leon V. Foster, of the Bausch and Lomb Optical Company, surveyed the steady development of the optical microscope as he has observed it and participated in it during the past 30 years. Harold Osterberg, of the Scientific Instrument Division of the American Optical Company, discussed in a scholarly fashion the somewhat academic subject of microscope imagery and its interpretations, progressing beyond the classical work of Airy and Abbe on the diffraction field resulting from a test object with periodic structure, and suggesting a new criterion for computing the resolving power of a lens. Peter Gray, of the Biology Department of the University of Pittsburgh, spoke on the optical microscope as seen by a user. In a good-humored blast at microscope manufacturers, he pointed out various items which he felt were worthy of improvement in the several standard types of commercial microscopes. It is this reporter's opinion that many of his criticisms were unduly severe and that solutions to his problems are already available, although the rhetorical technique of exaggeration for emphasis is not to be discouraged! John R. Loofbourow, of the Massachusetts Institute of Technology, spoke on the relatively new field of microspectroscopy in which, by a combination of techniques of microscopy and spectroscopy that are almost standard, analyses can be carried out on one-hundredth to one-thousandth the amount of material ordinarily needed—an absorption spectrogram can be obtained from a sample weighing as little as one-millionth of a gram. Among the techniques presently available in this new field are the use of fluorescence spectra, of emission spectra, and of absorption spectra in the ultraviolet, visible, and infrared regions. Receptors other than the photographic plate can be used—for instance, photomultiplier tubes in the ultraviolet and bolometers in the infrared. Single crystals of organic materials can be studied, as can very small sections of tissues, or solutions, by employing special specimen holders or cells. Prof. Loofbourow is interested in the extension of these techniques to the very low temperatures of liquid nitro-

en or even of liquid hydrogen or helium. L. Marton, f the National Bureau of Standards, gave a careful omparison and contrast of the capabilities of optical nd electron microscopes. Although electron microscopes ometimes achieve the resolution of details as small as 0 angstroms in dimension, they do suffer from several perational disadvantages of a practical nature.

The subject of phase contrast microscopy was dealt with in contributed papers by L. I. Epstein, of Bausch and Lomb, and by F. Zernike, C. P. Saylor, and A. T. Bryce, who suggested employing the great sensitivity of the human eye in hue discrimination in connection with the phase principle in what they called color phase contrast. This is reminiscent of the employment of the eye's color sensitivity in a color translation process for ultraviolet microscopy, which E. H. Land first announced in *Science* early this year (*Science*, April 15, pp. 371–4). Elkan R. Blout, of the Polaroid Corporation, described work under way in the field of infrared microscopy, wherein the apochromatic reflecting microscope objectives designed by David Grey of Polaroid and manufactured by Bausch and Lomb are combined in an ingenious fashion with a standard Perkin-Elmer infrared spectrometer to give infrared spectra of very small samples, such as single hormone crystals or single fibers far too small to be examined with standard infrared instruments.

The field of optics is so broad and has so many different aspects of a theoretical and applied nature, that in a three-day meeting a very large number of subjects are touched upon. Of interest to many were the papers on spectrochemical analysis and those on optical instrumentation techniques, such as new or improved light sources for interferometry, for ultraviolet microscopy, and for infrared spectroscopy; narrow-band interference filters with 80 percent peak transmission; infrared polarizers, which are efficient out to beyond a wavelength of two microns; the use of artificial sapphire in optical elements of complex lens systems; and new fluorescent and phosphorescent phenomena and their application. A. C. S. van Heel, of the Technical University of Delft, Holland, described in an invited paper the methods he developed during and after the war for using extremely simple equipment in making measurements of very high precision. The accurate establishment of angles to less than one-tenth second of arc, the measurement of optical flatness to one-hundredth of a wavelength of light, the alignment of ships' shafts in two dimensions, and the calibration of the vibration magnitude of a church tower when bells are ringing are all subject to handling by these very simple and direct means. In the invited paper which preceded the contributed program on infrared theory and applications, Donald F. Hornig, of Brown University, gave an elegant description of the use of infrared absorption spectra in the study of modes of molecular vibration and of the structure of the solid state. This relatively new technique is proving a powerful supplement to x-ray diffraction in unraveling the secrets of the solid state.

The biologist and the biophysicist found much to interest them at these meetings. George Wald, of the Biological Laboratories of Harvard University, presented a masterly paper which compared and contrasted the eye and the camera. He referred to the three mechanisms for minimizing the chromatic aberration of the eye—namely, the photopic sensitivity shift toward the red from the blue end of the spectrum, where the chromatic error is most troublesome; the strong absorption by the lens of the eye in the near ultraviolet; and the distribution of the yellow carotenoid pigment xanthophyll around the fovea. Dr. Wald recounted the experiments of Kuehne with animal and human eyes in obtaining "optograms," that is, in using the eye as a camera in fact, making on the retina of the eye a picture in terms of bleached and unbleached rhodopsin (visual purple) of an object recently viewed, and he dismissed again that attractive detective story solution wherein the murderer is identified by peering into the eyes of the dead victim. He closed this very interesting lecture by showing a print taken from a gelatin film in which the light-sensitive substance was not silver bromide but rhodopsin extracted from cattle retinas. The subjects of color measurement, color vision deficiency, and other phases of vision and physiological optics were explored in a number of contributed papers. S. Q. Duntley and N. A. Finkelstein, of the Massachusetts Institute of Technology, with Edward A. Edwards, of the Harvard and Tufts Medical Schools, reported on ultraviolet spectral reflectance measurements made on living human skin, and studies of the distribution over the body of ultraviolet-absorbing materials, such as hemoglobin, carotene, and melanin.

George R. Harrison was presented with the Frederic Ives Medal of the Optical Society at the annual dinner, and in his responding speech he sketched the various important stages in his 30 years of spectroscopic research, which started at Stanford University with measurements of the intensity of spectral lines and is now progressing at the Massachusetts Institute of Technology in the modification of one of Michelson's ruling engines for the ruling of a new type of grating called the echelle, which is halfway between a conventional diffraction grating and a reflection echelon. It is Dean Harrison's hope that, by employing careful interferometric control in the ruling of a thousand or so lines spaced a fraction of a millimeter apart, he will be able to achieve the spectroscopist's dream of a resolving power of one million. As a climax to his paper, he showed his audience the first spectrograms taken with an echelle grating.

This meeting marked the end of the two-year term of Rudolf Kingslake, of the Eastman Kodak Company, as the society's president, and his replacement by William F. Meggers, of the National Bureau of Standards.

Your correspondent, who attends both larger and smaller meetings than the one reported upon here, cannot refrain from commenting on the pleasantness and efficiency of a scientific meeting of about this size—a registration of perhaps 500, a total of 50 or 60 papers in three days of sessions, but never more than two sessions proceeding simultaneously and the entire group convening for each invited paper. Let us hope that the Optical Society does not emulate some of its sister societies by becoming so overwhelmingly successful and popular that the size of its meeting is greatly multiplied!

STANLEY S. BALLARD

About People

Carl Barnett Allendoerfer, professor of mathematics at Haverford College, Haverford, Pennsylvania, has been appointed visiting professor of mathematics at Massachusetts Institute of Technology for six months beginning in February, 1950. Dr. Allendoerfer, who has conducted research in the field of differential geometry and the connections between differential geometry and topology, was recently on leave at the Institute for Advanced Study at Princeton.

Vernon L. Mattson, former chief engineer of the Consolidated Feldspar Corporation, Trenton, New Jersey, has been appointed director of the Colorado School of Mines Research Foundation. The foundation, organized early this year, will conduct research in industrial mineralogy.

Benjamin M. Siegel has been appointed head of Cornell University's new Electron Microscopy Laboratory. Before going to Cornell, Dr. Siegel was in charge of the design and construction of a new type of electron microscope at the Research Laboratory of Electronics at Massachusetts Institute of Technology.

X. J. Musacchia, veteran of several arctic biological expeditions, has been appointed to the faculty of the Department of Biology at Saint Louis University. Dr. Musacchia will take part in research experiments in the university's Arctic Research Program.

Philip S. Jastram, formerly instructor in the Physics Department at the University of Michigan, has been appointed assistant professor of physics at Washington University, St. Louis.

William F. Bale, professor of radiation biology at the University of Rochester, has received a leave of absence to act as radiobiologist in the Division of Biology and Medicine of the U. S. Atomic Energy Commission. Dr. Bale will be responsible for the biological and health aspects of the AEC's waste disposal program.

Harry Huger Houston, former vice president of the Brooks Manufacturing Company, Knoxville, Tennessee, has joined the staff of Armour Research Foundation of Illinois Institute of Technology. Dr. Houston will conduct research in the Department of Ceramics and Minerals.

Frederick D. Rossini, chief of the Thermochemistry and Hydrocarbons Section of the National Bureau of Standards, has been appointed professor and head of the Chemistry Department at Carnegie Institute of Technology. The appointment will become effective July 1, 1950.

Visitors

Under sponsorship of the Microbiological Institute, **Sir Philip Manson-Bahr,** of London, will conduct a seminar on "Problems of Filariasis," to be held in Wilson Hall, Administration Building of the National Institutes of Health, Bethesda, Maryland, at 3 p.m. Thursday, December 1. Sir Philip, author of *The dysenteric disorders, The life and work of Sir Patrick Manson,* and the 7th to 12th editions of *Manson's tropical diseases,* is in the U. S. on his way to Fiji, where he will join his son in studies of filariasis.

Pablo Kleinman was a recent visitor at the U. S. Geological Survey in Washington. He has been assigned by the Chilean government to study water resource developments in this country. Another caller at the Geological Survey was **R. J. Bocaranda,** of Venezuela, who is interested in ground-water investigations.

Grants and Awards

The National Cancer Institute has awarded Public Health Service grants of $907,212 to aid clinical and laboratory research in nonfederal hospitals and universities in 21 states and the District of Columbia. Fifty-nine awards were renewals. A list of the new grants follows: Stanford University, *E. L. Tatum and A. C. Griffin,* $4,104, nitrogen-mustard carcinogenesis; University of California at Berkeley, *H. M. Evans,* $20,000, relation of the growth hormone to neoplasms; University of Southern California, *D. C. Pease,* $4,536, the electron microscopy of ultrathin tissue sections; Chicago Medical School, *Israel Davidsohn,* $13,198, natural and immune antibodies in inbred mouse strains with low and high tumor incidence; University of Illinois College of Medicine, *A. C. Ivy and Rhoda Grant,* $3,870, a study of the chemical and mechanical factors which may alter the normal growth pattern of the gastric mucosa; University of Southern Illinois, *C. C. Lindegren,* $5,000, genetics of yeast; Indiana University, *E. E. Campaigne,* $1,998, chemotherapy and carcinogens of the carbazole series; Harvard University, *F. J. Stare and R. Olsen,* $6,423, metabolism of tumor tissues; Detroit Institute of Cancer Research, *A. R. T. Denues,* $8,370, fine structure of chromosomes of normal and malignant cells; University of Michigan, *H. M. Pollard,* $5,000, nutritional and blood changes resulting from gastrectomy for gastric carcinoma, and further study of gastric cancer cells, and *A. B. Lerner,* $8,748, investigation of the biochemistry, development, and diagnosis of melanomas; St. Louis University, School of Medicine, *E. A. Doisy,* $4,000, metabolism of radioactive cortisone; University of Missouri, *M. N. Green,* $5,184, the effect of furan derivatives on the metabolism of bacteria and on the growth of transplanted tumors in mice; Albany Medical College, *A. W. Wright and J. M. Wolfe,* $7,344, further study of the etiology of spontaneous mammary tumors in the Albany strain of rats, with particular reference to the possible presence of a milk agent; Brooklyn College, *Irving Kaye,* $6,350, synthesis of N-(2-pyridyl)-substituted-α, β-diphenyl-ethylamines; Columbia University School of Dentistry, *B. M. Levy,* $3,000, a study to determine whether tumors can be experimentally produced in the mouth and lips of animals; Hickrill Chemical Research Foundation, Inc., New York City, *W. von E. Doering,* $10,000, synthesis of colchicine molecule for possible inhibitory cell activity; Memorial Hospital, New York City, *R. W. Rawson and H. J. Tagnon,* $8,910,

studies on the inactivation of estrogens and other steroids by liver tissue from cancer patients; Montefiore Hospital, New York City, *E. J. Baumann*, $5,616, selective filtration of the thyroid; University of Rochester, *H. L. Segal*, $4,000, the use of exchange indicator compounds for detecting achlorhydria without intubation; Ohio State University, *H. A. Hoster*, $1,660, studies on Hodgkin's disease and related conditions; University of Cincinnati, College of Medicine, *R. W. Vilter*, $7,895, the culture of human bone marrow in a synthetic medium; The Institute for Cancer Research, Inc., Philadelphia, *G. L. Miller*, $1,296, study of cancer tissue proteins; Wm. H. Singer Memorial Research Laboratory, Pittsburgh, *R. C. Grauer*, $6,350, the influence of various fractions of estrogens on biologic response; University of Pennsylvania, *Charles Breedis*, $5,346, blood supply and drainage of neoplasms in the liver and lung, and *D. W. Wilson and J. M. Buchanan*, $10,800, nucleic acid metabolism; Medical Branch, University of Texas, *G. Sinclair*, $3,240, study of the effects of small amounts of urethane on mice; Medical College of Virginia, *G. Z. Williams*, $7,800, study of purines and pyrimidines in nucleoprotein fractions of precancerous rat livers, and *N. F. Young*, $7,344, influence of certain proteins on the ability of the liver to destroy a carcinogenic agent, *p*-dimethylaminoazobenzene; University of Washington, *R. J. Blandau*, $4,968, study of experimentally produced endometrial polyps in guinea pigs; University of Wisconsin, *Charles Heidelberger*, $4,644, a study of metabolism of carcinogenic hydrocarbons labeled with radioactive carbon; Georgetown University Medical School, *C. F. Geschickter, M. M. Copeland, and Martin Rubin*, $5,000, therapeutic procedures for the retention and redistribution of radioactive phosphorus in patients with malignant disease; and George Washington University School of Medicine, *C. E. Leese*, $6,120, physiological effects of bacterial polysaccharides.

The William H. Nichols Medal of the New York section of the American Chemical Society has been awarded for 1950 to Oskar Wintersteiner, head of the Organic Chemistry Division at the Squibb Institute for Medical Research, New Brunswick, New Jersey. The medal was awarded to Dr. Wintersteiner "in recognition of fundamental contributions to the fields of insulin chemistry, steroid hormones, antibiotics, and alkaloids, and the first isolation in crystalline form of penicillin-G and streptomycin."

The Roscoe B. Jackson Memorial Laboratory for cancer research at Bar Harbor, Maine, received a $15,000 gift recently from the cancer fund of the national women's auxiliary of the Veterans of Foreign Wars. The funds will be used by the laboratory in the rebuilding of its library rooms, which were destroyed in a forest fire in 1947.

The Priz René Leriche of the International Society of Surgery has been awarded to Alfred Blalock, chief surgeon of The Johns Hopkins Hospital. He was honored for his contributions to vascular surgery.

The Sir Henry Wellcome Medal and Prize of the Association of Military Surgeons of the United States was awarded to Elliott Hurwitt for his essay, "A Blood Vessel Bank under Military Conditions," at the association's annual dinner on November 11.

Four grants totaling $15,000 have been awarded the **University of Illinois College of Medicine.** A $10,000 grant from the Pauline E. Reutinger Memorial Fund will be used by the Department of Pathology for studies in arteriosclerosis by Maurice Lev. The Department of Medicine received two Abbott Laboratories grants, one for $2,500 for a study of dietary therapy in liver disease, and a second of $500 for studies in anesthesia. Smith, Kline and French has made a $2,000 grant for the study of the effect of amines in experimental renal and other experimental hypertensions, to be undertaken by E. A. Ohler in the Department of Physiology.

The Damon Runyon Memorial Fund has presented checks totaling $101,000 to eight institutions for the support of cancer research: New York University-Bellevue Medical Center, University of Rochester School of Medicine, University of Notre Dame, Tulane University School of Medicine, University of Pennsylvania, Rutgers University-Presbyterian Hospital (in New Brunswick and Newark, New Jersey), Harlem Hospital, and the Jewish Hospital in Brooklyn. The memorial fund has allocated nearly $3,000,000 since it was set up in 1947.

Fellowship

A predoctoral research fellowship for the fundamental study of the chemistry of glycerides has been established at the University of Pittsburgh by Armour and Company, Chicago. The fellowship, extending over a period of three years, will be under the supervision of B. F. Daubert, research administrator, Department of Chemistry.

Meetings and Elections

The third annual American Medical Association clinical meeting will be held at the National Guard Armory in Washington, D. C. December 6–9. The meeting will be devoted to the problems of the general practitioner.

The 1949 annual meeting of the **American Physical Society** will be held, for the most part, in the buildings of Columbia University, in New York City, February 2–4, 1950. Titles and abstracts of all contributed papers must reach the APS by *December 9*. Further details can be obtained from Karl K. Darrow, Secretary, American Physical Society, Columbia University, New York 27, N. Y.

A national meeting on histochemistry will be held at the Department of Anatomy, University of Pennsylvania, Philadelphia, on March 25, 1950. Workers in the fields of anatomy, pathology, biochemistry, endocrinology, bacteriology, and other related sciences are

invited to attend. Further information can be secured from R. D. Lillie at the Laboratory of Pathology and Pharmacology, National Institutes of Health, Bethesda 14, Maryland.

The Indiana Academy of Science, at its annual fall meeting, elected the following officers for 1950: president, S. S. Visher, Indiana University; vice president, O. B. Christy, Ball State Teacher's College; secretary, W. A. Daily, Eli Lilly and Company; and treasurer, W. P. Morgan, Indiana Central College.

Officers for 1949–50 elected at the annual general meeting of **the Indian Association for the Cultivation of Science** were M. N. Saha, president; C. C. Biswas, first vice president; J. C. Ghose, second vice president; and P. Ray, honorary director.

Deaths

Frank B. Jewett, 70, president of the Bell Telephone Laboratories from 1925 until his retirement in 1940, died November 18, following an emergency operation. Dr. Jewett was responsible for developing the Bell Laboratories into one of the great research institutions of the world. In 1939 he was elected president of the National Academy of Sciences, the first scientist from the industrial field of research to win this distinction. Dr. Jewett was the recipient of many honors and was to have received the Hoover Medal at the January meeting of the American Institute of Electrical Engineers.

Francis E. Randall was killed in the November 1 airplane crash at Washington at the age of 35. Dr. Randall was in charge of research in physical anthropology at the Quartermaster Climatic Research Laboratory, Lawrence, Massachusetts.

Paul G. Heineman, 88, bacteriologist, died November 4 after an extended illness. Dr. Heineman retired as chief bacteriologist of Sterling Drug Company, Cook Laboratories, in 1939. Prior to his service with this company, he had been director of biological laboratories at the U. S. Standard Serum Company.

George Francis Eaton, 77, former curator of osteology and associate curator of vertebrate paleontology at Peabody Museum, Yale University, died at the home of his son in Mystic, Connecticut, on November 6. Dr. Eaton was a member of the Yale Peruvian Expedition to Machu Picchu in 1912.

William J. Humphreys, meteorological physicist at the U. S. Weather Bureau for thirty years, died in Washington on November 10. Dr. Humphreys, who was 87, was professor emeritus at George Washington University at the time of his death.

The U. S. Civil Service Commission has announced an examination for geologists to fill positions paying $3,100 and $3,825 a year in federal agencies, primarily in the Geological Survey and the Bureau of Reclamation, Department of the Interior, in the Bureau of Plant Industry and Soil Conservation Service, Department of Agriculture, and in Corps of Engineers, Department of the Army. Full information is given in announcement No. 199, available at any first- or second-class post office. Applications must be received by December 6, 1949, in the commission's Washington office.

Japanese scientists will soon be receiving isotopes under the foreign distribution program of the Atomic Energy Commission. Surveillance will be maintained by headquarters of the Supreme Commander for the Allied Powers, to assure the isotopes' safe and effective use for work in medicine and biology and research in the physical sciences. Japan is the first occupied country to be admitted to the program. The total number of foreign nations now participating is 30.

Recently Received—

The Role of Very Fine Mineral Matter in the Hot Water Separation Process as Applied to Athabaska Bituminous Sand. K. A. Clark and D. S. Pasternack. Report No. 53, Research Council of Alberta, University of Alberta, Edmonton, Canada. 15¢.

A Short Biography of Japanese Scientists, 1948. Vol. V-2, Metallurgy. Scientific Education Bureau, Ministry of Education, Tokyo, Japan.

Vampyroteuthis Infernalis Chun: an archaic dibranchiate cephalopod: II External Anatomy. Grace Pickford. Dana Report No. 32, 1949. Carlsberg Foundation, Oxford University Press, London. 1£.

Problems of Vole Populations in the Middle East. F. S. Bodenheimer. Research Council of Israel. Azriel Printing Works, Jerusalem. 250 mils.

Research in Review, 6th Report of the Sugar Research Foundation, Inc., 52 Wall Street, New York City.

Inventory of Published and Unpublished Sediment-Load Data in the U. S. Bull. 1. Soil Conservation Service, U. S. Department of Agriculture, Washington 25, D. C. On request.

Yeast of Tomorrow. Anheuser-Busch, Inc., St. Louis, Missouri. On request.

Catalogue of Birds of the Americas. Charles E. Hellmayr and Boardman Conover. Part I, No. 4, Zoological Series, Vol. XIII, Field Museum of Natural History. $4.00.

An Introduction to the Dynamics of Compressible Fluids, PB 97906. Library of Congress, Photoduplication Service, Publication Board Project, Washington 25, D. C. Photostat, $20.00; microfilm, $6.00.

Testing of Hydrometers. Elmer L. Peffer and Mary G. Blair. Circ. C477, Superintendent of Documents, U. S. GPO, Washington 25, D. C. 10¢.

U. S. Atomic Energy Commission Contracting and Purchasing Offices and Types of Commodities Purchased. U. S. GPO, Washington 25, D. C. 10¢.

Report of the Joint Committee on Atomic Energy. Pursuant to Public Law 585, 79th Congress. U. S. GPO, Washington 25, D. C. On request.